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Thijs M.H. Eijsvogels



Physiological demands of prolonged exercise

Science of the Nijmegen Four Days Marches

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Physiological demands of prolonged exercise

Science of the Nijmegen Four Days Marches

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Promotor

Prof. dr. M.T.E. Hopman

Copromotor

Dr. D.H.J. Thijssen

Manuscriptcommissie

Prof. dr. J.L.R.M. Smeets (voorzitter)

Prof. dr. H.A.M. Daanen (Vrije Universiteit, Amsterdam)

Prof. dr. H. Kuipers (Universiteit Maastricht, Maastricht)

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inlevere
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Total body fluid (42 L)

Intracellular
20.0 L

Extracellular

Interstitium
10.0 L

Plasma
3.2 L

Core body temperature

Distance
Grady
Laboratory

NaCl

Chapter 1

General introduction and outline of the thesis

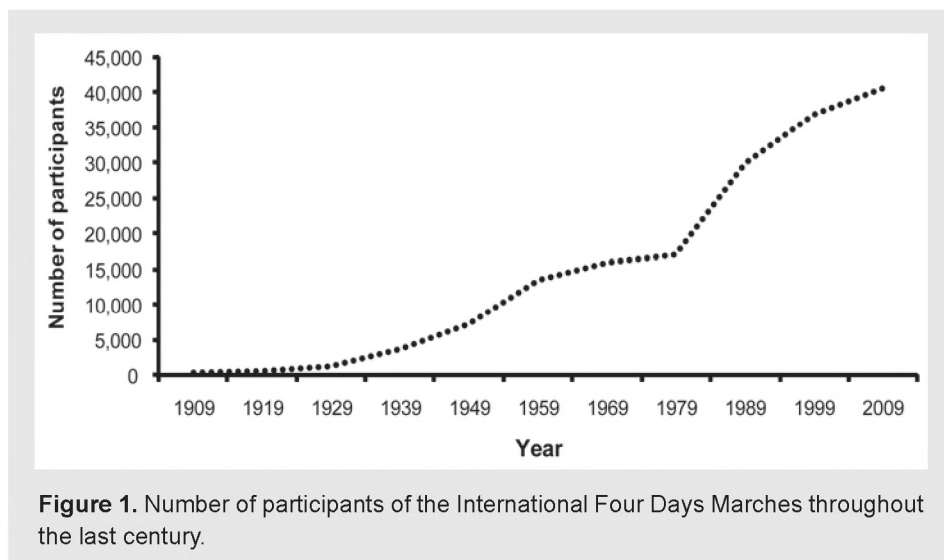


The relation between physical activity and health is well known and frequently described. The Greek physician Hippocrates (460 - 375 B.C.) already recognized this relationship: "All parts of the body, if used in moderation and exercised in labours to which each is accustomed, become thereby healthy and well developed and age slowly; but if they are unused and left idle, they become liable to disease, defective in growth and age quickly". Later, in the 2nd half of the 20th century, the first scientific evidence was collected during large epidemiological trials.¹⁻⁵ These studies showed a strong and inverse relation between physical activity and the development of cardiovascular diseases or overall mortality. Currently it has been well established that inactivity increases the risk to develop chronic diseases such as coronary heart disease, hypertension, stroke, osteoporosis, type 2 diabetes and cancer.⁶⁻¹⁰ In addition, physical inactivity leads to large socioeconomic costs and an enormous effect on health care resources.¹¹ In the Netherlands, 6 – 10% of the total health care budget is spend to treat patients with diseases that are caused by physical inactivity,¹² whilst annually 8000 deaths are due to an inactive lifestyle.¹³

In the past 20 years, health care workers and governmental organisations stimulated physical exercise in the general population.¹⁴ For example, the American College of Sports Medicine (ACSM) introduced the slogan 'Exercise is Medicine' to encourage exercise for a better health.¹⁵ Moreover, the ACSM and the American Heart Association developed a guideline for physical activity and public health.¹⁶ Around the same time, the 'Nederlandse Norm Gezond Bewegen' was introduced in The Netherlands after a consensus meeting of physicians and scientists in 2000.¹⁷ Both guidelines recommend that adults need to perform exercise at a moderate-intensity level for a minimum of 30 minutes per day on at least 5 days a week, or at a vigorous-intensity level for a minimum of 20 minutes a day on at least 3 days a week. Currently only 56% of the Dutch population meets the criteria of a physically active lifestyle.¹⁸

Walking is a common and popular activity for many individuals.¹⁹⁻²² One reason is that walking is easy accessible for subjects across a large age range, whilst exercise intensity is moderate.²³ The Netherlands has a good infrastructure for walking and many walking events are organized annually. Furthermore, walking is an effective and often prescribed therapy by physicians to improve health and/or cardiorespiratory fitness in patients with a large range of pathologies.²⁴⁻²⁶ Finally, walking exercise has deep roots in the evolution of humans and can be performed in numerous settings;^{27, 28} e.g. for transportation, during sports (e.g. golf, marching, etc.), in leisure time (e.g. dog walking, hill walking and hiking), and as a physical challenge (e.g. long distance walking).²⁹⁻³² The number of participants in organised walking marches increased rapidly during the last century (Figure 1).

During these popular, large scale athletic events, trained and untrained recreants walk for many hours under a wide range of ambient conditions. Although walking



on a regular basis has a harmless character and is related to health benefits, prolonged exercise may also lead to health problems. Previous studies indicated that athletes of endurance exercise events, such as a marathon or triathlon, can develop dehydration, hyperthermia and hyponatremia, whilst also elevated biomarkers of cardiac damage have been reported.³³⁻³⁸ However, little is known whether these problems also occur during prolonged walking exercise.

Current knowledge on the impact of walking exercise on physiological parameters is mainly based on case reports. A case series reported the occurrence of hyponatremia in seven students after recreational hiking.³⁹ Furthermore, two other papers describe the presence of exertional heat illness and hyponatremia in hikers under demanding conditions.^{40, 41} In addition, large scale health problems were reported during the 2006 edition of the world's largest walking event; the International Four Day Marches (see Box 1). With ambient temperatures above 33°C, numerous participants fainted, whilst many heat-related problems occurred, and 2 subjects died.⁴² The lack of knowledge about the physiological demands of walking, in combination with the major health problems during the Four Day Marches increased public awareness of the potential risks of prolonged walking. Therefore, more insight is required regarding physiological responses of participants of these walking marches. In this chapter I will review the physiological demands and responses during exercise in healthy athletes, but also potential disturbances of the thermoregulatory system, fluid- / sodium balance, and the cardiovascular strain.

Box 1. The International Four Days Marches

The Four Days Marches is an annual non-competitive walking event which is organized in Nijmegen, The Netherlands. It was founded in 1909 by the Dutch military, and is currently developed into the world's largest walking march with 45,000 participants every year. The Four Days Marches starts traditionally at the third Tuesday of July. Participants walk a minimum distance (30, 40 or 50 km) which is based on gender and age. At 04.00 hour in the morning participants are allowed to start, whilst they have to finish before 17:00 hour in the afternoon. Usually 10% of the participants drops out during the event, most of them on the second day.⁴³

Thermoregulation

The thermoregulatory system

In humans, body temperature comprises the temperatures of the core and shell. The core body temperature refers to the temperatures of the abdominal, thoracic and cranial cavities, whereas the shell temperature relates to the temperature of the skin and subcutaneous tissue. Our thermoregulatory system is a complex interaction between the central nervous system, the cardiovascular system and the skin. The thermoregulatory centre is located in the neurons of the preoptic area of the anterior hypothalamus of our brain, and this is the site where the core body temperature set-point is determined.⁴⁴ The thermosensitive neurons in the hypothalamus receive information regarding core and shell temperatures from central and peripheral skin receptors, respectively. Since the thermoregulatory system acts as a negative-feedback system, this thermo-afferent information is compared to the set-point and any difference between afferent temperature signals and set-point temperature induces a thermoregulatory response to limit the divergence (Figure 2). For example, heat loss mechanisms are activated during a rise in core body temperature, whilst a decline in core body temperature results in the activation of mechanisms that conserve or produce heat.⁴⁵

We are able to regulate our core body temperature over a wide spectrum of ambient conditions within a relatively narrow range around $\sim 37^{\circ}\text{C}$.^{46, 47} Several internal and external factors are able to affect our thermoregulatory system, leading to acute or sub-acute changes in the core body temperature. For example, a distinct circadian rhythm in core body temperature can be observed in humans. After a nadir in the morning between 04:00 – 06:00 hour, core body temperature steadily rises and peaks 1-4 h before habitual bedtime.⁴⁸ The amplitude of this diurnal variation is 0.5°C in healthy individuals.⁴⁹ In addition,

age influences the core body temperature. Previous studies revealed that elderly have lower baseline core body temperatures than young adults.⁵⁰⁻⁵² Furthermore, the menstrual cycle significantly alters core body temperature. In premenopausal women, core body temperature is shifted upward by approximately 0.4°C in the luteal phase compared with the follicular phase.^{53, 54} Taken together, temperature set-point is depend on age, sex, and time of the day, whilst the variation in core body temperature is maintained within small limits.⁵⁵

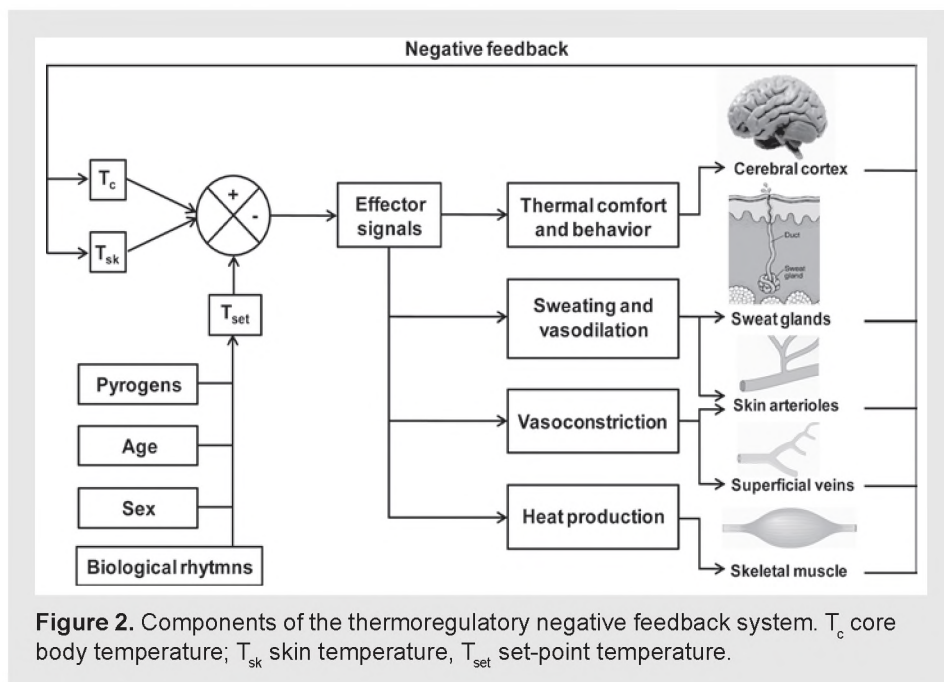


Figure 2. Components of the thermoregulatory negative feedback system. T_c core body temperature; T_{sk} skin temperature, T_{set} set-point temperature.

Thermoregulation during exercise

The oxidation of substrates during physical exercise importantly impacts the core body temperature as only 20% is used for muscle power, whilst the majority results in heat production (80%). As the metabolic heat production can increase 20 times above baseline values during strenuous exercise, humans importantly depend on heat loss mechanisms during these demanding conditions. Heat transfer between the body and the external environment occurs through 4 distinct processes; 1) conduction – transfers heat from warmer to cooler objects through direct physical contact, 2) convection – heat transfer to or from the body to surrounding moving fluid or air, 3) radiation – the energy is transferred to or from an object or body via electromagnetic radiation from higher to lower energy surfaces, 4) evaporation – heat transfers via the vaporization of sweat; the effectiveness of this process is dependent on the water saturation of the air and the velocity of the moving air.

Despite optimization of heat dissipating mechanisms, heat can accumulate in the body under certain circumstances. This is typically observed during exercise, where the production of metabolic heat eventually leads to an increase in core body temperature. Previous studies showed that participants of (half) marathons,⁵⁶⁻⁵⁹ or Ironman triathlons,^{60, 61} demonstrate a wide range in finish core body temperature (38.1°C to 39.9°C). The magnitude of this temperature response is primarily related to the duration and intensity of exercise (e.g. metabolic heat production). However, also ambient conditions (air temperature, humidity) and the fluid balance (dehydration) contribute to the thermoregulation and the increase in core body temperature.³⁸ Whilst thermoregulation is well described during marathons, only a few studies examined thermoregulation during walking. Ainslie *et al.* showed that core body temperature increased during a 12 km hill walk from 37.0°C to a maximum of 38.8°C.⁶² They also report that this increase is most likely due to the thermal insulation from the protective clothes worn by the hill walkers (leading to a decreased heat loss capacity). Other studies that assessed thermoregulatory responses during walking are restricted to laboratory tests and predominantly focus on hypothermia during cold stress.⁶³⁻⁶⁵

Thermoregulatory disorders

The thermoregulatory system is importantly challenged during exercise. The combination of prolonged or high intensity exercise with a warm and/or humid environment can lead to the development of heat illness. The traditional classification of heat illness has 3 categories: heat cramps, heat exhaustion, and heat stroke.^{38, 66} These conditions are discussed below, including the potential health problems associated with these thermoregulatory disorders.

Heat cramps

Heat cramps are one of the earliest indications of heat illness and arise in the form of muscle spasms or muscle cramps. This typically results after excessive heat exposure, inadequate fluid and electrolyte intake and/or profuse sweating. Accordingly, the muscles may demonstrate spasms, experienced as painful contractions, often resulting in the inability to continue activity for a short time.^{67, 68} Sodium loss is thought to play a significant role in the etiology of heat cramps,⁶⁹ but no conclusive evidence is present at the moment.

Heat exhaustion

Heat exhaustion is the inability to continue exercise with a core body temperature that ranges between 37°C and 40°C.⁷⁰ Heat exhaustion often occurs in hot and humid conditions, and is characterized by heavy sweating, malaise, fatigue and dizziness. Also nausea, vomiting, headache, fainting, weakness and cold or clammy skin may be observed.⁷⁰ As this condition worsens, it is difficult to distinguish heat exhaustion from exertional heat stroke without measuring rectal temperature. However, critical to the diagnosis of heat exhaustion is a normal mental activity and a stable neurologic status.⁷¹

Heat stroke

The most threatening type of heat illness is exertional heat stroke. This condition is characterized by a core body temperature above 40°C, in combination with a central nervous system disturbance (irritability, ataxia, confusion, coma).^{38, 66, 71} Exertional heat stroke occurs when the temperature regulation system is overpowered by excessive endogenous heat production or inhibited heat loss in challenging environmental conditions. Heat stroke is potentially life threatening and can be fatal unless promptly recognized and treated. Signs and symptoms are often nonspecific and include disorientation, tachycardia, vomiting, seizures, loss of balance and coma. In a later stage rhabdomyolysis, circulatory failure, multiple organ failure, and disseminated intravascular coagulation may occur and could lead to the death of an athlete.^{38, 66, 71} Currently, exertional heat stroke is the third leading cause of death in athletes in the United States following cardiac disorders and head and neck trauma.^{71, 72} The only effective therapy to treat collapsed athletes is rapid and aggressive whole body cooling.^{38, 73}

Hypothermia

At the other end of the thermoregulatory spectrum, prolonged exposure to cold stress can lead to hypothermia. This condition is defined as a decline in core body temperature below 35°C.⁷⁴ Hypothermia is usually the result of prolonged exposure to cold air, immersion in cold water, or due to impaired thermal insulation of (wet) clothing. The classification of hypothermia and the degree of physical complaints is thereby inversely related to the core body temperature.⁷⁵ Whilst mild hypothermia (32°C – 35°C) results in shivering, tachycardia and apathy, subjects with moderate hypothermia (28°C – 32°C) demonstrate arterial dysrhythmias, bradycardia, hyporeflexia and a decreased level of consciousness. When the core body temperature drops below 28°C (severe hypothermia), apnea, coma, cardiorespiratory failure may occur, which could eventually lead to the death of an individual.⁷⁴⁻⁷⁶

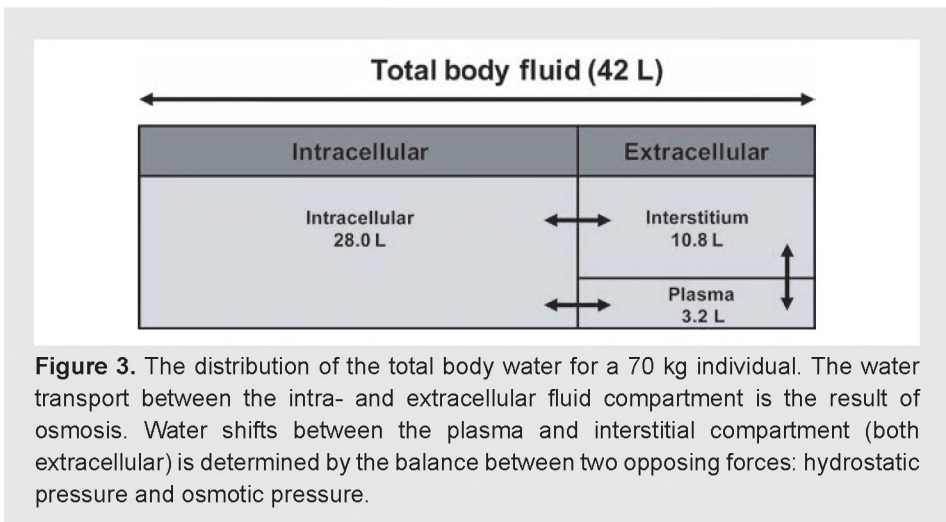
Fluid- and sodium balance

Fluid balance

For an average adult, total body water represents ~60% of their body weight.⁷⁷ This water is distributed into intracellular (65%) and extracellular (35%) fluid compartments. The extracellular fluid compartment is further divided into the interstitial and plasma spaces.⁷⁷ For example, a 70 kg individual consists of ~42 liter body water, with 28 liter in the intracellular fluid compartment, 3.2 liter in the plasma and 10.8 L in the interstitium (Figure 3). These volumes are not static, but represent a net effect of the dynamic exchanges of fluid homeostasis.

The regulation of the fluid balance is a continuous process and is subject to a large number of variables that can cause a disturbance of the fluid balance. For

example, water can be lost from the body through the skin, feces, lungs, and kidneys, causing an increase of the osmolarity and a decrease in the circulating blood volume. Osmoreceptors in the hypothalamus detect the increase in osmolarity, which will stimulate thirst perception and the secretion of arginine vasopressin.^{78,79} Vasopressin will cause water reabsorption in the kidney, which contributes to preserve fluid volume by preventing a further decrease in blood volume. The thirst stimulus will cause the subject to ingest fluid (if available), which causes the blood volume and osmolarity to restore to baseline values. In addition, if plasma volume shows a decrease of more than 10%, changes in arterial pressure are detected by the baroreceptors in the carotid sinus, aortic arch, left atrium and great pulmonary veins, resulting in a thirst stimulus and in the release of additional arginine vasopressin secretion to further attempt to restore plasma osmolarity.⁷⁸ Whilst these processes ensure fluid retention during fluid losses, the opposite responses can be expected during fluid overloading (i.e. a decrease in arginine vasopressin concentration and increase in urine secretion).



Sodium balance

Sodium is the major determinant of serum osmolarity, which is an essential regulator of arginine vasopressin secretion and thirst perception. Under normal conditions, the plasma sodium concentration is regulated between 135 and 145 mmol/L. The regulation of sodium, however, must be integrated with regulation of plasma volume, because changes in water volume alone have diluting or concentrating effects on the bodily fluids. Aldosterone, a steroid hormone that is produced by the adrenal cortex, plays a central role in the sodium regulation. Changes in plasma osmolarity are directly sensed in the adrenal cortex, resulting in aldosterone inhibition or secretion during an increased or decreased osmolarity,

respectively. During dehydration, more water than sodium is lost from the human body resulting in an increase in plasma osmolarity. The inhibition of aldosterone release causes less sodium to be reabsorbed in the distal tubule. Simultaneously, the increased osmolarity also causes a stimulation of arginine vasopressin, leading to water conservation in the kidneys. The net effect is decreased amount excreted urine, with an increase in the osmolarity of the urine. These reactions are complementary to restore the sodium concentration and plasma osmolarity.

Fluid balance during exercise

In humans, fluid is primarily lost through evaporation, which is necessary to compensate for the enormous production of heat during exercise. The increased muscle workload during exercise leads to a markedly increased heat production. More than 80% of this heat is dissipated through evaporative heat loss mechanisms, making this the primary method of heat removal from the body.⁸⁰ In addition to exercise intensity (and therefore to the amount of muscle workload), sweat rate is also dependent on environmental conditions (temperature, humidity and wind velocity), clothing, acclimatization, body mass index and gender.⁸¹⁻⁸⁴ Sweat rates for endurance athletes range from 0.5 to 1.5 L/h with the capability of reaching amounts larger than 3.0 L/h under highly demanding conditions.^{85, 86} Fluid is also lost in small amounts through the respiratory system as water is evaporated from the airways.⁸⁷ However, this small amount of water loss is roughly compensated by metabolic water production during oxidation of substrates in the process of muscle contractions.^{88, 89} Taken together, various mechanisms can contribute to fluid losses during exercise, with evaporation of sweat as the largest contributor.

Fluid- and sodium disturbances

As it is difficult to maintain a stable homeostasis during prolonged exercise, athletes are prone to develop fluid and sodium disturbances. However, limited data is available related to fluid and sodium responses during walking exercise. In the classic study of Adolph and colleagues, sweat rate was assessed in soldiers who walked in the Nevada desert.⁹⁰ They found a variation between 300 and 1000 mL/h, depending on activity, clothing and time of day. Furthermore, they reported fluid losses up to 7.4% of the baseline body mass after an 8 hour desert hike in absence of water ingestion.⁹⁰ In a more recent field study, body mass losses of 3.4% were reported after prolonged marching with a backpack in soldiers that were allowed to drink ad libitum.⁹¹ These studies suggest that the fluid and electrolyte balance are challenged in subjects that perform prolonged walking exercise. Accordingly, participants of prolonged walking events may be prone to develop dehydration, hypernatremia or hyponatremia.

Dehydration

Insufficient fluid intake to compensate for fluid losses during exercise can lead to the development of dehydration. This is defined as a body mass loss of 2% or more, which is estimated to be equal to a total body water loss of ~3%.^{34, 81, 92, 93}

Dehydration of an athlete typically occurs during prolonged exercise in temperate and warm ambient conditions.^{34, 92, 94-97} The impact of dehydration on exercise performance and the thermoregulatory system is widely studied as (severe) dehydration may eventually contribute to (serious) health problems in athletes. Dehydration causes a reduction of plasma volume as well as a reduction of stroke volume. Although a temporarily increase in heart rate can preserve cardiac output and hence performance levels, the heart(rate) fails to compensate when dehydration progresses. The increased heart rate and diminished cardiac output cause an impaired aerobic exercise performance in athletes. Pitts *et al.* was one of the first to demonstrate that a progressive loss of body water during prolonged exercise resulted in an elevated heart rate (~17%), consequently leading to an impaired endurance walking performance (20%–60% decreased walking duration).⁹⁸ Moreover, dehydration may also result in an exaggerated increases in core body temperature to a given exercise stimulus. Several studies showed that the magnitude of dehydration directly relates to a higher core body temperature during exercise within subjects.^{96, 99, 100} This emphasizes that, in addition to the impaired exercise performance, dehydration may increase the risk of developing heat related illnesses.^{38, 81, 101}

To prevent dehydration, subsequent health problems, and to preserve performance levels, several guidelines for fluid replacement during exercise have been developed.^{81, 102-105} The most important message is that athletes should attempt to prevent excessive fluid losses. A simple but useful tool for athletes is to monitor body mass before and after exercise. Whereas most guidelines state that body mass losses of 2% or more should be avoided,^{81, 102-104} one guideline promotes drinking according to the dictates of thirst.¹⁰⁵ Although these guidelines provide useful recommendations for athletes, most athletes experience difficulty changing their normal drinking behavior and/or meet these criteria to preserve body weight during a competitive race. Therefore, dehydration is still frequently observed during endurance exercise events.

Hypernatremia

Exercise in humans lead to a substantial loss of fluid through sweating to promote heat loss. Due to this mechanism, important changes in electrolyte balance in the body are observed. Sweating also leads to a loss of sodium, the most important mineral that contributes to electrolyte balances. Sweat is a hypotonic fluid, which means that humans loose relatively more fluid than sodium when sweating.¹⁰⁶ Therefore, prolonged exercise performance combined with (too) little fluid replacement, especially when performed under conditions that induce excessive sweat loss, is associated with an increase in plasma sodium levels (i.e. hypernatremia).^{107, 108} Hypernatremia is defined as a plasma sodium concentration of ≥ 145 mmol/L. Whilst mild levels of hypernatremia do not lead to (serious) clinical symptoms, acute and large levels of hypernatremia (>158 mmol/L) are associated with hyperpnea, restlessness, lethargy and even coma.¹⁰⁹

Hyponatremia

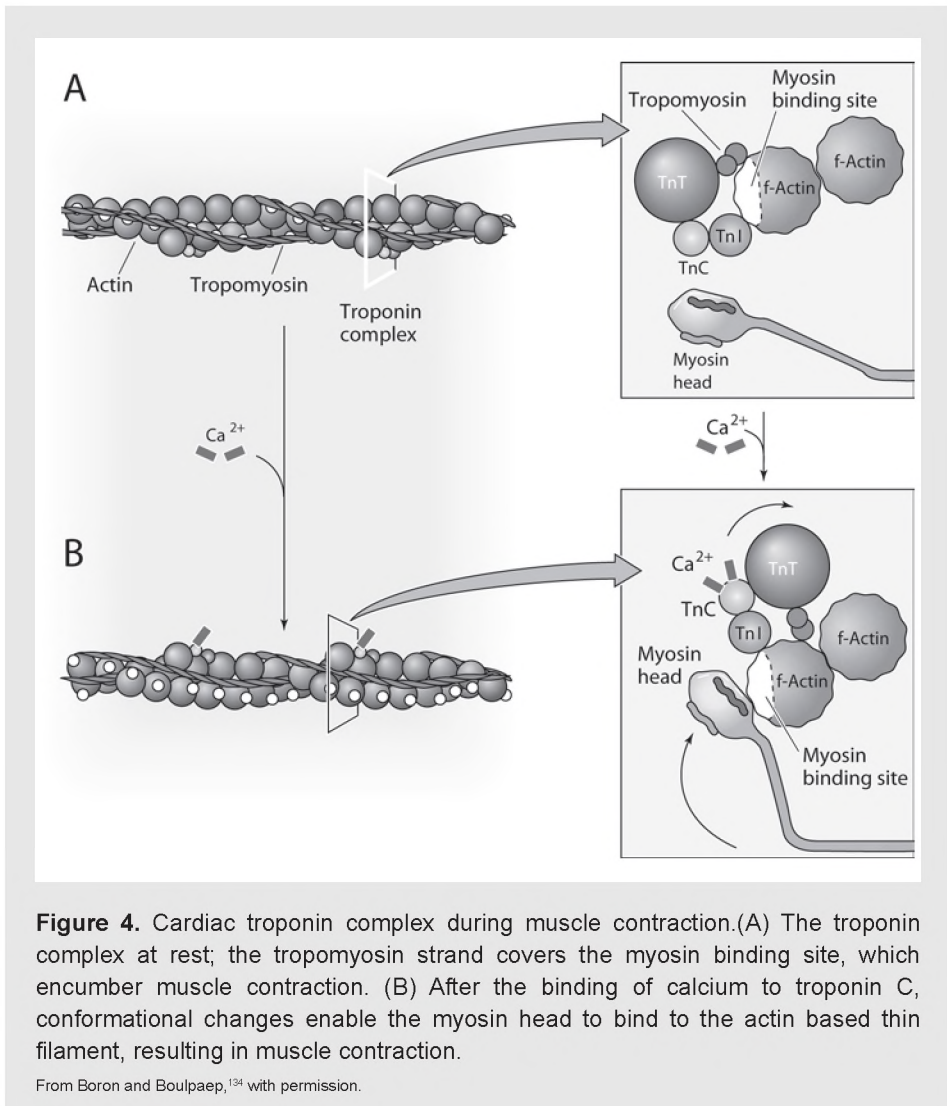
Exercise may also lead to the potentially lethal condition of hyponatremia, which is defined by a plasma sodium concentration of 135 mmol/L or lower.¹¹⁰ The low sodium concentration is caused by dilution due to an increase in total body water relative to the amount of total body exchangeable sodium. The primary etiological factor is abnormal fluid intake (water or sports drinks) in excess of fluid losses, often induced by ignorance of fluid replacement guidelines.^{111, 112} The onset of symptoms usually occurs at a sodium concentration below 130 mmol/L, with nausea, vomiting and headache.^{36, 40, 113} As the level of hyponatremia continues to decrease, more serious signs and symptoms can develop as a result of cerebral edema, including alteration of mental status, seizures, coma and death.^{110, 114-116} Athletes with a symptomatic hyponatremia should be immediately treated with hypertonic saline to reduce brain edema.^{117, 118} Together with heat stroke, exercise associated hyponatremia is the most dangerous disorder for athletes.

Cardiovascular strain

Endurance exercise has a marked impact on the heart of an athlete. After the onset of exercise, the sympathetic nervous system is stimulated and catecholamines (epinephrine and norepinephrine) are released. These hormones stimulate the pacemaker potential of the sinoarterial node. The subsequent increase in heart rate and contractility causes an elevated cardiac output, which strongly correlates with the oxygen demand of skeletal muscles. The effects of (prolonged and chronic) exercise on the heart have been examined extensively. One of the most characteristic adaptations of the heart comprises the remodeling process induced by systemic exercise, which is commonly referred to as the “athlete’s heart”. This morphological change is generally regarded as a benign increase in cardiac mass that represents a physiological adaptation to the increased cardiac load by exercise training.¹¹⁹⁻¹²² Since a few years, studies have also described the acute effects of exercise on the heart. Specifically, the release of cardiac troponin, a marker for cardiac damage, during prolonged exercise has been matter of debate. Although the benefits of moderate and high-intensity exercise are well established,^{16, 123, 124} these findings suggest that exercise might be associated with cardiac damage.

Cardiac troponin

The principal components of the contractile apparatus of striated muscle (sarcomere) are the actin-based thin filament, the myosin-based thick filament, tropomyosin and the troponin complex (Figure 4).¹²⁵ During muscle contraction, the actin and myosin filament slide over each other. This action requires calcium (Ca²⁺), which is released from the sarcoplasmic reticulum upon calcium influx resulting from depolarization of nerve fibres. The troponin complex is tadpole-shaped, composed of 3 subunits (troponin C, troponin T and troponin I), and



plays a central role in muscle contraction.^{126, 127} Troponin T structurally binds the troponin complex via troponin C to tropomyosin. Binding of Ca^{2+} , after release of this substance from the sacroplasmic reticulum, to troponin C results in conformational changes and increased affinity of troponin C for troponin I. Troponin I then releases its inhibitory function on actomyosin ATPase, which leads to ATP hydrolysis and access of the myosin head to the actin binding site.¹²⁸ Subsequently, the muscle fiber can contract. While troponin C is identical in both

skeletal and cardiac muscle, troponin I and T have specific isoforms for skeletal and cardiac muscle. The highly specific cardiac isoforms of troponin I and T, make these proteins suitable for detecting cardiac damage as circulating troponin I and T are extremely low in healthy subjects, but markedly increase after cardiac damage. Since several studies showed the superiority of cardiac troponin above creatine kinase MB-isoenzyme (the gold standard for cardiac damage in earlier days),^{129, 130} cardiac troponin was established as the standard biomarker for the diagnosis of myocardial infarction world wide.¹³¹⁻¹³³ However, it was recommended that only values above the 99th percentile of a healthy population, in combination with an acceptable assay precision (coefficient of variation $\leq 10\%$), were used as a cut-off for the definition of acute myocardial infarction.¹³¹⁻¹³³ Nowadays, all high sensitive assays meet these criteria, thereby enabling clinicians and scientists to detect cardiac troponin levels across a broad range of subjects.

Exercise-induced cardiac troponin elevation

Although an increase in cardiac troponin levels is a strong marker for cardiac damage and is used in the diagnosis of a myocardial infarction, elevated circulating troponin concentrations have also been reported after prolonged high-intensity exercise.^{37, 135-140} Young, healthy, asymptomatic athletes demonstrated post-exercise troponin levels, which are well above the clinical cut-off value that is used for the diagnosis of acute myocardial infarction. Whilst these elevated levels of cardiac troponin suggest that strenuous physical exertion may result in myocardial injury, none of these athletes demonstrated (patho)physiological evidence or clinical signs of (ischaemic) myocardial damage. Hence, it was discussed whether these exercise-induced elevations of cardiac troponins might be a physiological response to strenuous exercise itself rather than a pathological response to myocardial damage.^{141, 142} Current observations, however, are restricted to high intensity endurance exercise only. It is not known whether other types of exercise, including moderate intensity or a shorter duration exercise type, also increase post-exercise troponin levels.

Another important aspect of troponin release relates to the subjects characteristics. In contrast to many studies that demonstrated elevated troponin levels in participants of cycling, running or triathlon events, only little is known which subjects are at risk. Most studies have an observational character, assessed only a few subject parameters and included a small number of subjects. These limitations hindered them from additional analysis to identify subjects or groups at risk for large increases in post-exercise cardiac troponin. Shave *et al.* combined the results of 26 studies in a meta-analysis.¹⁴³ They found that relatively heavy individuals competing in endurance events (e.g. marathon running) demonstrated larger troponin elevations than other athletes. Insight into who might be at increased risk for larger exercise-induced troponin release is potentially useful for clinicians when evaluating a cardiac troponin test in athletes/recreants who performed prolonged exercise. To further elucidate which parameters contribute

to the magnitude of the exercise induced elevations of troponin levels, future studies should have a prospective character, include large groups of participants and should address multiple exercise-related parameters.

Outline of the thesis

Aim of the present studies

The popularity of walking has increased markedly in the past decades, resulting in a heterogeneous population that participates in organized marches. Nowadays, young and old, men and women, trained and untrained, obese and lean, and subject with and without pathology or medication walk under different ambient conditions. Interestingly, previous case reports demonstrate that walking is associated with an increased risk for disturbances of the thermoregulation, fluid- and sodium balance and the cardiovascular system.³⁹⁻⁴² Despite the moderate-intensity level of walking exercise, the prolonged exposure to this type of exercise as well as the heterogeneity in health status of the participants may have contributed to the relatively large number of health problems. Much of our current knowledge about the physiological demands during prolonged exercise is based on athletes performing high-intensity exercise, such as a marathon or triathlon. This systemic bias makes extrapolation of current knowledge from the physiological demands and potential risks in endurance exercise to prolonged walking extremely difficult. Therefore, the general aim of this thesis is to gain better insight into the physiological demands of prolonged walking, specifically focusing on the thermoregulation, fluid- and electrolyte balance and cardiac troponin release. In addition, we will identify specific groups at increased risk for these changes, which will enable the organization of walking events to improve the safety and health of their participants.

Studies that investigate the effects of walking are limited, and are restricted to studies that included young and healthy military recruits only. Therefore, our first target was to determine the physiological responses of prolonged walking. In *Chapter 2* we have included a heterogeneous group of participants, whilst we assessed the influence of walking on the core body temperature, fluid- and sodium balance.

Fluid disturbances are frequently reported during prolonged exercise. Previous studies suggest that sex may impact fluid loss and fluid intake during high-intensity endurance exercise, such as a marathon. For example, higher sweat rates in men compared to women,^{83, 84} and sex differences in the changes in sodium concentration after exercise have been reported.^{110, 144} To examine potential differences between men and women during walking exercise, we examined the impact of sex on fluid balance responses in *Chapter 3*. We hypothesized that men are more prone to the development of dehydration than women.

Another parameter that may have a significant effect on the physiological responses to exercise is obesity. Due to the larger body surface area and a greater number of sweat glands, obese subjects typically have larger fluid losses than lean controls during exercise.⁸² Furthermore, it is known that obese subjects demonstrate an altered thermoregulatory response,¹⁴⁵ with body mass index being positively related to the risk of developing heat disorders in military recruits.^{146, 147} Since it is unknown whether obesity in the general population is related to the development of fluid and sodium imbalances during prolonged exercise, we compared the physiological responses of prolonged walking exercise of lean, overweight and obese subjects in *Chapter 4*.

In the scientific world, it is widely acknowledged that exercise can induce elevations in cardiac troponin levels. However, previous studies are restricted to strenuous, high-intensity endurance exercise only. As walking leads to a significant increase in cardiac work, which may be present for up to 13 h during a the Four Days Marches, we determined the effect of prolonged walking on cardiac troponin levels in *Chapter 5*. In addition we assessed the impact of body mass index on cardiac troponin release after exercise in *Chapter 6*. This is of particular importance since exercise training is routinely prescribed to individuals with obesity,¹⁴⁸⁻¹⁵⁰ with a preference for moderate-intensity exercise that can be performed for prolonged periods (e.g. walking or cycling). In addition, previous studies showed that obesity and body mass are related to elevated troponin levels at baseline and during exercise.^{143, 151}

Exercise leads to elevations in cardiac troponin levels, sometimes even above the clinically relevant cut-off levels for the diagnosis of a myocardial infarction. This makes the interpretation of a troponin assay extremely difficult, especially in athletes who are brought to the hospital after performing a strenuous exercise bout. *Chapter 7* deals with this problem by presenting and discussing 3 distinct cases of athletes who demonstrate elevation in post-exercise troponin levels. A detailed discussion and comparison between these cases may help clinicians and laboratories to improve the clinical management of athletes who demonstrate troponin levels above the clinical cut-off value.

Finally, the current knowledge on the physiological demands of prolonged walking is reviewed and discussed from previous studies and available data from this thesis in *Chapter 8*. In addition, we examined the impact of prolonged walking on a single day (which is consistently used in the previous chapters) versus multiple days. Furthermore, the influence of ambient conditions on physiological responses is presented and discussed.

Experimental model

To assess the physiological aspects of prolonged walking, we have deliberately chosen to perform field experiments instead of laboratory testing. First, field

experiments represent real life situation in which we are interested. By collecting data in these conditions, results and conclusions can be directly applied to these events. Second, using a field study does not ignore the important impact of convection, pacing strategy and fluctuating ambient conditions as most laboratory studies do. Third, performance of a field study enabled us to test all subjects on the same days, ensuring similar weather conditions in all subjects. In this thesis, measurements are performed in participants of the International Four Days Marches in Nijmegen (Box 1). Subjects were randomly selected, while they were counterbalanced for age, gender and walking distance. This enabled us to include a representative sample of the average population of a walking event.

Measurement Techniques

Core body temperature

The temperature of blood in the pulmonary artery is considered as the best representation of the average internal temperature of the human body.¹⁵² As this site is not easy accessible, core body temperature is often measured at the mouth, axilla, tympanic membrane, esophagus or rectum. The esophageal temperature at the level of the left atrium provides the closest agreement with central blood, while it also responds rapidly to temperature changes.¹⁵³ Although it is generally considered as the most accurate technique, its invasive nature limits the practical use of this method. Alternatively, many (laboratory) studies use rectal temperature to monitor changes in core body temperature during exercise. As these temperatures respond slowly to changes in exercise intensity, this method is considered as an acceptable measurement during steady-state conditions only.^{152, 154}

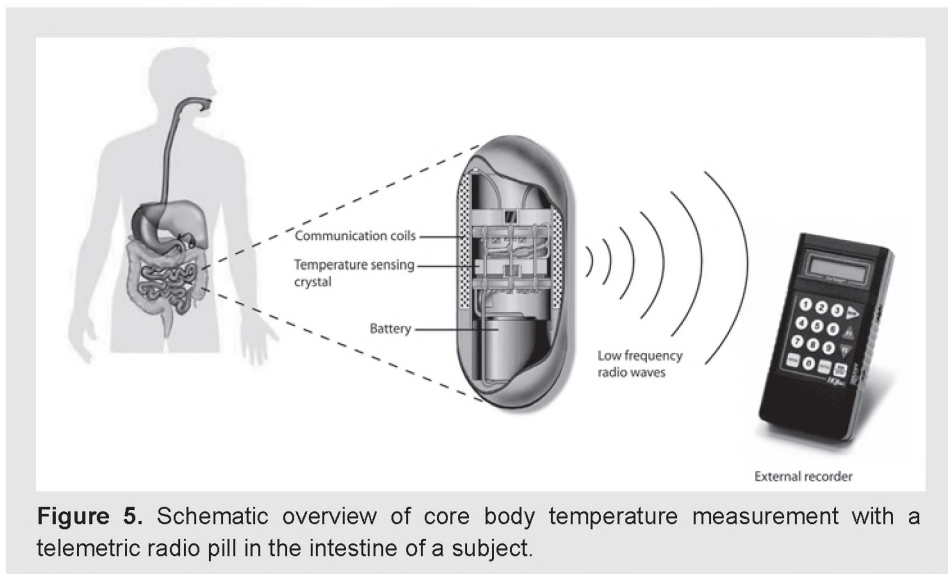


Figure 5. Schematic overview of core body temperature measurement with a telemetric radio pill in the intestine of a subject.

Core body temperature can also be assessed using a telemetric radio pill (CorTemp™ system, HQ Inc, Palmetto, USA). This ingestible temperature pill was first described in 1961,¹⁵⁵ and further developed at the Johns Hopkins University in collaboration with the Applied Physics Laboratory of the NASA. The result is a 20 x 10 mm capsule with a telemetry system, micro battery and a quartz crystal temperature sensor. The crystal sensor vibrates at a frequency relative to the temperature of the surrounding substance. This temperature radio-signal is transmitted through the body, which is picked up by an external recorder (Figure 5). Several studies showed that this technique is safe and reliable, whilst it gives an accurate measure of core body temperature.¹⁵⁶⁻¹⁶² Although fluid and food intake may acutely influence core body temperature measurements, a recent study demonstrated that this bias can be ignored when the pill was swallowed 4 – 8 hours before assessment.¹⁶³ Taken together, the telemetric radio pill is an excellent technique to determine (changes in) core body temperature in outdoor conditions.

Fluid and sodium balance

The assessment of hydration status of an athlete is difficult since there is no gold standard.¹⁶⁴⁻¹⁶⁷ Although the determination of the total body water by isotope dilution is regarded as the best option, this technique is expensive and not applicable in field situations. Alternatively, it has been suggested to measure multiple parameters that, when combined, provide a comprehensive comparison of an individual's fluid balance.¹⁶⁵ Therefore, we collected a series of accepted and reproducible parameters to determine the hydration status of subjects in our studies:

Body mass changes. Before the start and directly after exercise, body mass was assessed to the nearest 0.1 kg (Seca 888 scale, Hamburg, Germany). The relative change in body mass (in %) between both measurements was calculated, whilst dehydration was defined as a body mass loss of 2% or more.^{34, 81, 92, 168, 169} A recent study reinforced that this approach is valid to examine the hydration status of an athlete, and that body mass changes can be used as a surrogate marker for changes in total body water.¹⁷⁰

Plasma sodium concentration. To determine the levels of plasma sodium concentration (Rapidpoint® series, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.), 2 ml of venous blood was drawn after 5 minutes rest in an upright position. An increase or decrease of the sodium level was associated with a net water loss and water gain, respectively.^{107, 108} Hyponatremia and hypernatremia were defined as a plasma sodium concentration of ≤ 135 mmol/L and ≥ 145 mmol/L, respectively.^{109, 110}

Plasma volume changes. Using hematocrit (Rapidpoint® 400, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.) and hemoglobin (HemoCue

AB, Ängelholm, Sweden) levels at baseline and directly post-exercise, we calculated relative changes in plasma volume (%) according Dill and Costill's equation: $\Delta PV (\%) = 100 * ((Hb_{baseline} / Hb_{post-exercise}) * (1 - Hct_{post-exercise} * 0.01)) / (1 - Hct_{baseline} * 0.01) - 100$.¹⁷¹

Urine specific gravity. A 5 mL urine sample at baseline and directly after the finish was used to determine urine specific gravity (Clinitek Status® Analyzer; Siemens Healthcare Diagnostics, Tarrytown, USA). While urine specific gravity is usually regulated between 1.013 and 1.029 g/mL, values can change as a consequence of dehydration (USG > 1.030) or overhydration (USG < 1.012).^{102, 164}

We also collected additional information regarding fluid intake and fluid excretion of our subjects. Fluid intake was assessed using a diary. Subjects were allowed to drink ad libitum, whereas they registered the time and amount (standard sized cups, bottles) of their fluid intake from 12 hours preceding the exercise until the end of the experiment. Before the start of the Four Days Marches, all subjects received written and individual oral instructions regarding the registration of their fluid intake. This approach is in line with previous studies and frequently applied in field-based studies.^{41, 112, 144} In addition, urine was collected into a specialized collecting bag (Roadbag/Ladybag; KETs GmbH, Köln, Germany) to determine the amount of urine output during exercise. Bags were collected and urine volume was weighed at the laboratory within 0.1 g accuracy (PT 1500; Sartorius AG, Göttingen, Germany). Furthermore, sweat rate (mL/h) was calculated by combining body weight, fluid intake and urine output data using the formula: $\text{Sweat rate (mL/h)} = (\text{pre-exercise body weight} - \text{post-exercise body weight} + \text{fluid intake} - \text{urine output}) / \text{exercise duration}$.¹⁰² Although this formula ignores respiratory and gastrointestinal fluid losses,^{89, 166} it is plausible that the metabolic water production in the muscles compensates for the respiratory fluid losses,^{88, 89} whilst gastrointestinal losses are normally negligible (i.e. 100 mL/day).¹⁶⁶

Cardiovascular strain

All subjects wore a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland) to monitor heart rate during walking exercise. Mean heart rate during exercise was calculated as the average heart rate, excluding the values derived directly before the start and after the finish. The maximal predicted heart rate was subsequently calculated according to the formula of Tanaka *et al.* ($208 - 0.7 * \text{age}$).¹⁷² Using these values, we determined exercise intensity of prolonged walking in each individual (average heart rate / maximal predicted heart rate * 100%).

To determine cardiac troponin concentrations, 10 ml venous blood was drawn from an antecubital vein at baseline and directly after finishing. Whole venous blood was collected in serum-gel vacutainer tubes and allowed to clot for ~45 min. After centrifugation, serum was aliquoted, frozen and stored at -80°C for

later analysis. Post-experiment analysis was performed on a single day using the same calibration and set-up to minimize variation. Cardiac troponin I was analyzed using a normal- and a high-sensitive troponin I assay (Immulite 2500 (Chapter 5) or Centaur TnI-Ultra (Chapter 6), respectively, Siemens Healthcare Diagnostics, Breda, the Netherlands). The assay imprecision of the normal troponin I assay was 8.1% at 0.08, 8% at 2.3 µg/L and 7.9% at 29.1 µg/L, with a detection limit of 0.1 µg/L. The high sensitive assay had an imprecision of 10% at 0.03 µg/L, 5.3% at 0.08 µg/L and 3.0% at 27.2 µg/L, with a detection limit of 0.006 µg/L. A troponin I value of 0.2 µg/L (normal assay) and 0.04 µg/L (high-sensitive assay) were used as the clinical cut-off values for myocardial infarction.¹⁷³

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Click to - and produce > 100 Pa

Total body fluid (42 L)

Intracellular

Interstitium 10.0 L

Extracellular

Interstitium 10.0 L

Plasma 3.0 L

Cell body temperature

Distance
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Core body temperature, fluid intake and sodium balance during prolonged walking

Adapted from: Nederlands Tijdschrift voor Geneeskunde, 2008;152:1571-8



Abstract

Background. Walking exercise is a popular physical activity and is often prescribed by physicians to improve health. Since recent case reports have reported the presence of heat-related illnesses and sodium disturbances, more insight is needed into the physiological responses of prolonged walking. Therefore, the purpose of this study was to determine the impact of prolonged walking exercise on core body temperature, fluid and sodium balance in a large heterogeneous group of subjects.

Methods. 66 volunteers (22-81 years), counterbalanced for gender and age, participated in the Nijmegen Four Days Marches and walked 30-50 km at a self-selected pace. Heart rate and core body temperature were recorded every 5 km. Subjects reported fluid intake, whilst baseline and post-exercise levels of plasma sodium were determined.

Results. Subjects performed walking exercise for ~8.5 h at a speed of 4.7 km/h with a relative exercise intensity of 68% of the maximum predicted heart rate. Core body temperature increased from 37.3°C to 38.0°C ($p < 0.001$), and significantly correlated with exercise intensity ($r = 0.38$, $p < 0.005$). An average fluid intake of 301 ± 155 mL/h was reported. Plasma volume decreased with -4.3%, whilst sodium levels decreased from 142.3 to 141.2 mmol/L. In total 15% of our subjects finished with a sodium disturbance (2% hyponatremia and 13% hypernatremia).

Conclusion. Prolonged walking exercise is associated with an increase in core body temperature, but also with the presence of post-exercise sodium disturbance (15%). Although these findings suggest that prolonged walking exercise represents a substantial physiological strain, this was well tolerated under temperate climate conditions.

Introduction

Prolonged walking exercise has deep roots in the evolution of humans.¹ Being the primary method for transportation for the hunter-gatherer, walking is nowadays a popular physical activity²⁻⁴ and is often prescribed by physicians to improve health.⁵⁻⁷ Long distance hiking and (multiple days) marches (50-80 km) are frequently performed by trained and untrained recreants.⁸ Although commonly regarded as a safe type of exercise, recent case studies have described the presence of heat-related illness⁹⁻¹¹ and sodium disturbances^{9, 12} in healthy individuals after completing prolonged walking exercise.

Previous studies that examined the impact of prolonged walking exercise on physiological responses are restricted to healthy, (highly) trained young subjects.¹³⁻¹⁷ Since walking exercise is nowadays commonly performed by a heterogeneous group of participants, extrapolation of previous findings to the general public is limited. Therefore, the purpose of this study was to assess core body temperature, fluid intake, and plasma sodium concentration in a large heterogeneous group of participants that walked 30, 40 or 50 km on a single day, to improve our understanding of the physiological demands of prolonged walking exercise. We hypothesize that the core body temperature increases in all subjects, whilst the magnitude of this response will be related to the exercise intensity. Furthermore, we expect that walking alters the fluid and sodium balance, but these values will be maintained within normal limits.

Methods

Subjects

Sixty-six subjects (22 - 81 yrs) volunteered to participate in our study. Subjects with chronic (inflammatory) bowel problems were excluded from participation, since such problems are a contra-indication for the use of the telemetric temperature sensor. Subjects were counterbalanced for gender and age and walked 30, 40 or 50 km. The Medical Ethical Committee of the Radboud University Nijmegen Medical Centre approved the study and all subjects gave their written informed consent prior to participation. This study was conducted in line with the Declaration of Helsinki.

Experimental set-up

All subjects participated in the International Four Days Marches. Subjects reported twice to our laboratory, which was located at the start/finish area. Baseline measurements were performed on the day preceding the start. Thereafter, subjects walked 30 km (n=20, 50% men), 40 km (n=25, 56% men) or 50 km (n=21, 48% men) at a self selected pace. During exercise, heart rate and core body temperature were measured every subsequent 5 km, and all subjects

registered their fluid intake using a diary. Immediately after finishing, all baseline measurements were repeated.

Measurements

Subject characteristics. Body mass (Seca 888 scale, Hamburg, Germany) and height were measured. Resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min seated rest. All subjects completed a health questionnaire.

Walking characteristics. A bi-axial accelerometer (SenseWear Pro3, BodyMedia Inc, Pittsburg, USA) was placed around the right upper arm to examine exercise duration and energy expenditure. This activity monitor has been validated in free-living situations in previous studies.¹⁸⁻²⁰

Core body temperature. To monitor core body temperature, a portable telemetry system was used (CorTemp™ system, HQ Inc, Palmetto, U.S.A.), which is a safe and reliable method to examine core body temperature at rest and during physical exercise.^{21, 22} Subjects ingested an individually calibrated telemetric temperature sensor on the evening preceding the event, to avoid interaction with fluid ingestion during testing.²³ Prior to the start, 3 measurements were obtained, with core body temperature being calculated as the average of the 3 consecutive measurements. This procedure was repeated every 5 km along the route. The single highest value of these measurements was presented as peak core body temperature.

Heart rate. A chest band (Polar Electro Oy, Kempele, Finland) was used to examine heart rate, which was measured simultaneously with core body temperature using the same data recorder. Mean heart rate during exercise was calculated as the average heart rate, excluding the values derived directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate ($208 - 0.7 * \text{age}$).²⁴

Fluid intake. Written and individual oral instructions regarding the registration of fluid intake was provided. Subjects drank ad libitum, whereas they registered the time and amount (standard sized cups, bottles) of their fluid intake.

Blood analysis. After 5 minutes of seated rest, 2 ml of blood was drawn from the antecubital vein. Blood samples were directly analyzed from the collecting syringe for plasma levels of sodium and hematocrit levels using a Rapidpoint® 400 system (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.). Hemoglobin was immediately determined using a B-Hemoglobin analyzer (HemoCue AB, Ängelholm, Sweden). Hyponatremia and hypernatremia were

defined as a plasma sodium concentration of ≤ 135 and ≥ 145 mmol/l, respectively.²⁵
²⁶ Relative changes in plasma volume were calculated from blood hematocrit and hemoglobin concentrations using Dill and Costill's equation.²⁷

Ambient temperature. Dry bulb, wet bulb, and globe temperatures were measured every 30 minutes throughout the experiment using a portable climate monitoring device (Davis instruments inc., Hayward, U.S.A.) positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula: $WBGT = 0.1 (T_{\text{dry bulb}}) + 0.7 (T_{\text{wet bulb}}) + 0.2 (T_{\text{globe}})$.²⁸

Table 1. Subject characteristics and details about pathology classified by walking distance.

	Overall (n=63)	30 km (n=18)	40 km (n=25)	50 km (n=20)
Demographic characteristics				
Male : female (n)	34 : 29	10 : 8	14 : 11	10 : 10
Age (years)	56 \pm 17	70 \pm 6	54 \pm 19.1	47 \pm 11.1
Height (cm)	173 \pm 9	169 \pm 9	176 \pm 8.1	174 \pm 10
Weight (kg)	74.9 \pm 13.6	70.6 \pm 10.7	74.7 \pm 12.3	79.1 \pm 16.6
Body mass index (kg/m ²)	24.8 \pm 2.8	24.7 \pm 2.2	24.0 \pm 2.6	26.0 \pm 3.3
Blood pressure				
Systolic (mmHg)	144 \pm 19	148 \pm 23	146 \pm 17	137 \pm 17
Diastolic (mmHg)	86 \pm 10	87 \pm 10	85 \pm 8	85 \pm 11
MAP (mmHg)	105 \pm 12	107 \pm 14	105 \pm 10	103 \pm 12
Resting heart rate (bpm)	74 \pm 12	72 \pm 11	75 \pm 12	74 \pm 13
Health status				
Hypertension	15 (24%)	7 (39%)	5 (20%)	3 (15%)
Hypercholesterolemia	9 (14%)	2 (11%)	2 (8%)	5 (25%)
Cardiovascular disease	7 (11%)	3 (17%)	2 (8%)	2 (10%)
Asthma	4 (6%)	1 (6%)	3 (5)	0 (0%)
Diabetes	2 (3%)	0 (0%)	2 (8%)	0 (0%)
Other	6 (9%)	5 (28%)	0 (0%)	1 (5%)

[†] statistically different from 30 km group; BMI, body mass index

Statistics

Values are presented as means with standard deviation, unless indicated otherwise. The level of statistical significance was set at $p < 0.05$. Using the SPSS statistical software package (SPSS Inc., Chicago, U.S.A.), we examined differences across distance groups (30, 40 or 50 km) using a one-way analysis of

variance (ANOVA). Paired t-tests were used to examine the difference between pre- and post-exercise parameters. Correlations between measured physiological parameters were assessed using a Pearson's correlation coefficient.

Results

Three subjects did not finish due to joint related problems and were excluded from further analysis. Mean distance trained in the year prior to the event (self reported) was 434 km (0 - 3000 km), while 92% reported to meet the ACSM guidelines for physical activity (≥ 5 times a week, ≥ 30 minutes moderate-intensity exercise). Subjects in the 30 km group were significantly older ($P < 0.001$) than in the other 2 groups (Table 1). Body weight, BMI, blood pressure and resting heart rate were comparable across groups. 45% of our subjects used medication (predominantly antihypertensives (15%), anticoagulants (8%) and statins (6%)).

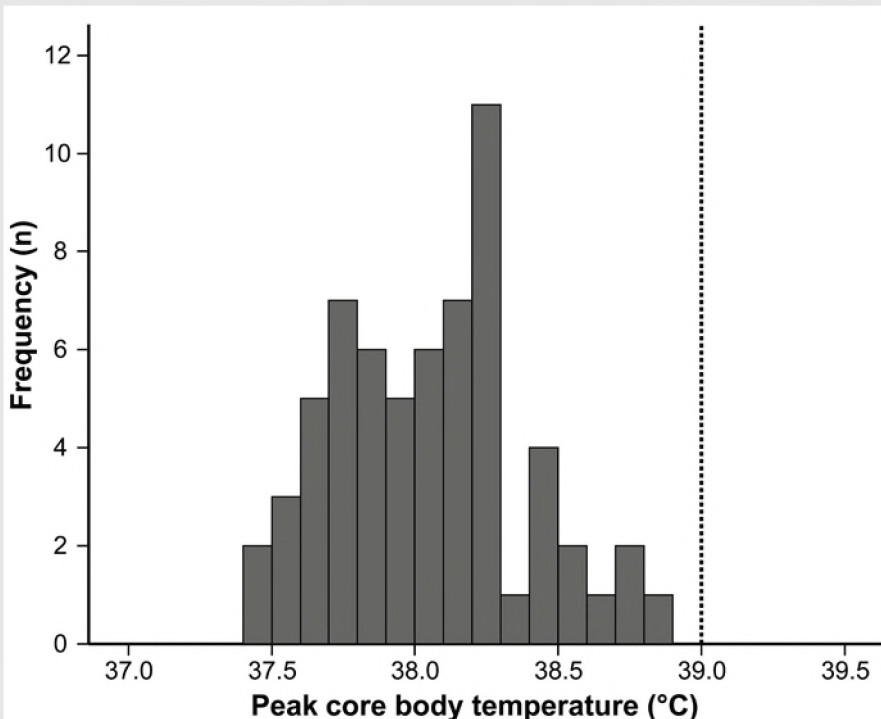


Figure 1. Frequency distribution of peak core body temperature in subjects that perform 30, 40 or 50 km walking exercise (n=63). The dotted line represents the threshold for hyperthermia.

Core body temperature

WBGT increased from 15.4°C in the early morning to a maximum of 22.3°C in the afternoon. Average walking duration and corresponding walking speeds differed across groups ($p < 0.001$, Table 2). Core body temperature increased to an average peak of 38.0°C ($p < 0.001$, Table 2), whilst the peak core body temperature showed values ranging from 37.4°C to 38.8°C (Figure 1). In parallel, heart rate during exercise was 115 beats per minute, which relates to an exercise intensity of 68% and did not differ across groups (Table 2). Exercise intensity and core body temperature were significantly correlated (Figure 2).

Table 2. Exercise characteristics, fluid and sodium balance classified by walking distance.

	Overall (n=63)	30 km (n=18)	40 km (n=25)	50 km (n=20)
Exercise characteristics				
Exercise duration (hh:mm)	8:37 ± 1:37	7:06 ± 0:41 _{2,3}	8:10 ± 1:00 _{1,3}	10:33 ± 0:45 _{1,2}
Speed (km/h)	4.7 ± 0.6	4.3 ± 0.4 ^{2,3}	5.0 ± 0.7 ¹	4.8 ± 0.4 ¹
Baseline T _c (°C)	37.3 ± 0.4	37.2 ± 0.5	37.3 ± 0.4	37.3 ± 0.5
Peak T _c (°C)	38.0 ± 0.3	38.0 ± 0.3	38.1 ± 0.4	38.1 ± 0.3
Exercise intensity (%)	68 ± 9	65 ± 9	69 ± 8	71 ± 10
Energy expenditure (kcal)	3542 ± 1123	2478 ± 387	3308 ± 760	4710 ± 781
Fluid and sodium balance				
Fluid intake (L)	2.6 ± 1.6	2.0 ± 0.8 ³	2.4 ± 0.9	3.4 ± 2.4 ¹
Fluid intake (mL/hour)	301 ± 155	276 ± 97	303 ± 120	321 ± 225
Fluid intake (mL/kg/hour)	4.0 ± 1.8	3.9 ± 1.5	4.1 ± 1.6	4.0 ± 2.4
Baseline sodium concentration (mmol/L)	142.3 ± 2.0	142.0 ± 2.6	142.6 ± 1.8	142.3 ± 1.6
Finish sodium concentration (mmol/L)	141.2 ± 2.9	141.3 ± 3.1	142.2 ± 3.2	140.1 ± 2.0
Delta sodium concentration (mmol/L)	-1.1 ± 2.7	-0.7 ± 2.9	-0.5 ± 3.0	-2.2 ± 1.7
Hypernatremia (%)	8 (13%)	2 (11%)	6 (25%)	0 (0%)
Hyponatremia (%)	1 (2%)	1 (6%)	0 (0%)	0 (0%)
Hematocrit (L/L)	43 ± 4	43 ± 3	44 ± 4	43 ± 4
Hemoglobin (mmol/L)	8.7 ± 1.1	8.8 ± 0.9	8.9 ± 1.2	8.4 ± 1.0
Calculated plasma volume change (%)	-4.3 ± 9.2	-3.4 ± 8.8	-6.1 ± 9.0	-3.1 ± 10.0

^{1,2,3} statistically different from 30 km, 40 km or 50 km group respectively; T_c, Core body temperature

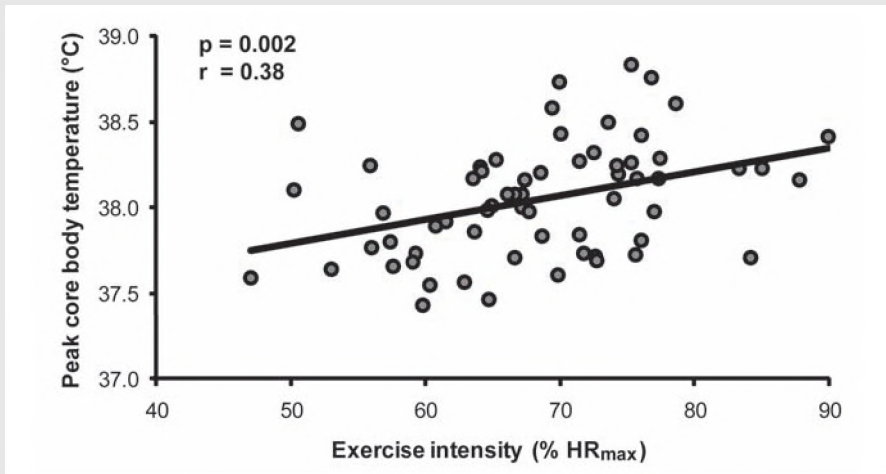


Figure 2. Correlation between exercise intensity and peak core body temperature during prolonged walking exercise (n=63).

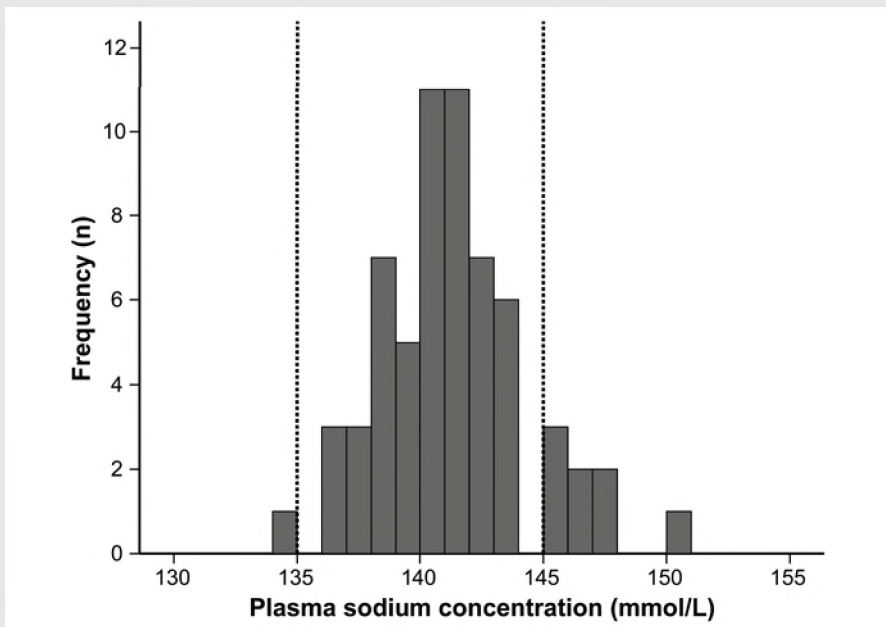


Figure 3. Frequency distribution of plasma sodium concentration after prolonged walking exercise (n=63). The dotted lines represents hyponatremia (<135 mmol/L) and hypernatremia (>145 mmol/L).

Fluid intake and sodium balance

Fluid intake differed significantly across the 3 distance groups (Table 2). However, after correction for exercise duration and/or body mass, no differences were found across groups (Table 2). Post-exercise plasma sodium concentration was lower compared with baseline ($P < 0.001$), with a prevalence of 2% for (asymptomatic) hyponatremia and 13% for (asymptomatic) hypernatremia (Figure 3). Characteristics of subjects with hypo-, normo- or hypernatremia are presented in Table 3. Plasma volume decreased with -4.3% after exercise (Table 2).

Table 3. Characteristics of subjects with hypo-, normo-, and hypernatremia.

	Hyponatremia (n=1)	Normonatremia (n=54)	Hypernatremia (n=8)
Subject characteristics			
Age (years)	77	54 ± 16	64 ± 15
Gender (% men)	0%	49%	100%
BMI (kg / m ²)	27.2	24.9 ± 2.8	25.2 ± 2.1
Exercise characteristics			
Speed (km / h)	3.5	4.7 ± 0.6	4.9 ± 0.5
Increase in T _{ce} (°C)	1.1	0.8 ± 0.5	0.8 ± 0.3
Maximum T _{ce} (°C)	37.7	38.1 ± 0.3	37.9 ± 0.2
Exercise intensity (% HRmax)	59	69 ± 10	66 ± 6
Fluid and sodium balance			
Fluid intake (L)	4.1	2.7 ± 1.7	2.0 ± 0.9
Fluid intake (mL / h)	475	307 ± 162	253 ± 101
Fluid intake (mL / kg / h)	6.8	4.1 ± 1.8	3.2 ± 1.5
Plasma volume changes (%)	2.1	-2.5 ± 11.6	-7.0 ± 9
Baseline sodium concentration (mmol/L)	142.6	142.3 ± 2.0	143.8 ± 1.4
Finish sodium concentration (mmol/L)	134.8	141.2 ± 2.9	146.7 ± 1.7
Increase in sodium concentration (mmol/L)	-7.8	-1.1 ± 2.7	2.8 ± 1.8

T_{ce}, Core body temperature

Discussion

Despite the popularity of walking in leisure time or as physical exercise, little is known about the impact of prolonged walking exercise on physiological characteristics. We found that, under moderate ambient conditions, prolonged walking substantially increases heart rate and core body temperature. Nonetheless, these values were maintained within normal limits during 9 hours of walking exercise. Moreover, a large variation in fluid intake and changes in plasma sodium concentration were observed. As a result, 15% of our subjects demonstrated a disturbance of the sodium balance. This study shows that prolonged, moderate-intensity walking exercise is well tolerated by subjects of various ages and health conditions, despite significant changes in heart rate, core body temperature and sodium balance.

Walking exercise was performed at 68% of the maximum predicted heart rate, indicating a moderate to strenuous activity which was maintained for ~8.5 hours. Subsequently, core body temperature increased with 0.7°C to a peak value of 38.0°C. This finding correlates well with observations during an Ironman triathlon, where a slightly higher increase in core body temperature (+1.0°C) and slightly higher exercise intensity was found (83%).²⁹ Although absolute work rate is significantly different between prolonged walking exercise and an Ironman marathon, the relative work intensity seemed to correlate well with the increase in temperature. Indeed, exercise and core body temperature were significantly correlated within our subjects (Figure 2). This indicates that prolonged walking exercise represents a considerable physiological strain on the human body, leading to an increase in core body temperature that matches well with exercise intensity.

Maintaining a stable fluid balance is important to regulate the electrolyte homeostasis. Given the marked differences in fluid intake between subjects, one may suggest that some individuals were at risk to develop sodium disturbances. We found that 15% of the total group of participants finished with hyper- or hyponatremia. Although excessively high or low levels of plasma sodium concentrations are potentially harmful, we found no clinical problems in our subjects. Because sweat is hypotonic, an increase in sodium concentration during exercise suggests a net fluid loss.³⁰ The larger decrease in plasma volume in subjects with hyponatremia reinforces this finding (Table 2). The presence of hyponatremia may therefore also relate to dehydration. As this condition can seriously affect exercise performance,^{31, 32} and the health status of an athlete,^{33, 34} future studies should assess the incidence of dehydration during prolonged walking. Particular attention should be paid to potential sex differences, since all hyponatremic subjects were men. The higher sweat rate in men compared to women is likely to contribute to this finding.^{35, 36}

Another important observation of this study is that one subject finished with hyponatremia. In accordance with previous studies and case reports, our subject reported a high rate of fluid intake.³⁷⁻³⁹ Although caution must be taken when discussing this subject, it is interesting to note that this subject is female and demonstrated a relatively low speed and an increase in plasma volume. These parameters were previously identified in subjects that participate in high-intensity endurance races.^{38, 40, 41} Physicians and organizations of walking events must therefore be aware of the presence of this sodium disturbance, as the routine administration of iso- or hypotonic fluids to collapsed participants on the presumption that a subject is dehydrated or hyperthermic may be catastrophic.³⁷

Clinical relevance. Large inter-individual variations were observed regarding fluid-intake, and (changes in) plasma sodium concentration. For example, plasma sodium concentration was regulated within safe limits in subjects that reported a fluid intake of 2.5 and 10.5 liter (140 and 141 mmol/l, respectively). This emphasizes that general recommendations regarding volumes and frequency of fluid intake are difficult and may even jeopardize individuals for fluid and electrolyte disturbances (e.g. dehydration or hyponatremia). Alternatively, subjects that participate in prolonged exercise events should be stimulated to determine their body weight before and after training sessions. The assessment of changes in body mass is a simple and clinically relevant strategy to determine dehydration (weight loss) or overhydration (weight gain). Subsequently, participants can easily estimate their personal fluid needs under different circumstances.

In conclusion, prolonged moderate-intensity walking exercise is well tolerated by subjects of various ages and health conditions. A modest increase in core body temperature is observed, which is significantly related to exercise intensity. More importantly, a large group of subjects (15%) demonstrated sodium disturbances. Given the strong interaction between the fluid and sodium balance, these data indicate that participants of walking events may also be prone for dehydration. Future studies should identify specific groups at risk for fluid and sodium disturbances during these popular walking exercise events.

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Total body fluid (42 L)

Intracellular

Interstitium

Extracellular

Interstitium

Plasma

Cell body temperature

Distance
Grady
Interstitium

NaCl

Chapter 3

Sex difference in fluid balance responses during prolonged exercise

Thijs M.H. Eijssvogels
Ralph R. Scholten
Noortje T.L. van Duijnhoven
Dick H.J. Thijssen
Maria T.E. Hopman

In revision



Abstract

Maintaining a proper fluid balance is important during exercise since athletes are prone to develop dehydration during exercise. Although several factors may regulate the fluid balance, little is known about the role of sex during prolonged moderate-intensity exercise. Therefore, we compared body mass changes and fluid balance parameters in men *versus* women in a large heterogeneous group of participants during prolonged exercise. Ninety-eight volunteers, walked 30-50 km at a self-selected pace. Exercise duration (8h32min) and intensity (69% HR_{max}) were comparable between groups. Men demonstrated a significantly larger change in body mass than women (-1.6% *versus* -0.9%, respectively, $P < 0.001$) and a higher incidence of dehydration (defined as $\geq 2\%$ body mass loss) compared to women (34% *versus* 12%, respectively, Odds Ratio=4.2, 95% CI=1.1-16.7). Changes in blood sodium levels were significantly different between men (+1.5 mmol/L) and women (-0.4 mmol/L), whilst 27% of the men *versus* 0% of the women showed post-exercise hypernatremia (sodium levels ≥ 145 mmol/L). Moreover, men demonstrated a significantly lower fluid intake (2.9 ml/kg/hr), and higher fluid loss (5.0 ml/kg/h) compared to women (3.7 and 4.8 ml/kg/h, respectively). Taken together, our data suggests that men and women demonstrate different changes in fluid balance in response to a similar bout of exercise.

Introduction

Imbalance between fluid intake and fluid loss during prolonged exercise may increase the risk for development of dehydration. Previous laboratory studies showed that substantial fluid/body mass losses were related to an impaired exercise performance^{1, 2} and to a larger increase in core body temperature.^{1, 3} Accordingly, dehydration may enhance the development of hyperthermia, heat-exhaustion and heat stroke.^{4, 5} Warm and humid conditions, high intensity exercise and/or prolonged exercise increases the risk of a mismatch between fluid intake and fluid loss and, thereby, the potential development of dehydration.^{6, 7}

Sex may impact fluid loss and fluid intake. Indeed, men have higher sweat rates which might lead to more fluid loss during exercise compared with women.^{8, 9} Previous studies also showed that men have higher plasma sodium levels and a higher prevalence of hypernatremia than women after prolonged exercise,^{10, 11} which suggests a larger fluid loss in men. In contrast, it is also reported that women have an increased risk for overdrinking, which could lead to exercise-associated hyponatremia.¹² Whilst these observations suggest that men are more prone to dehydration than women, little is known about a potential sex difference during prolonged, moderate-intensity exercise.

The purpose of this study, therefore, was to examine markers of dehydration during prolonged moderate-intensity exercise in men *versus* women in a large heterogeneous group of participants. Markers of dehydration include body mass changes, a $\geq 2\%$ decrease in body mass after finishing,^{6, 13} sodium levels and plasma volume changes after exercise. We hypothesize that men have a higher incidence of dehydration and will develop larger changes in markers of (de)hydration (e.g. sodium levels and plasma volume changes) than women. Furthermore, we aimed to identify factors (subject and race characteristics) that may contribute to the potential sex differences.

Methods

Subjects

Ninety-nine participants (21 - 82 years, 57 men and 42 women) volunteered to participate in this study (Table 1). Subjects with chronic (inflammatory) bowel problems were excluded from participation as a contra-indication for the use of the telemetric temperature sensor. The Medical Ethical Committee of the Radboud University Nijmegen Medical Centre approved the study and all subjects gave their written informed consent prior to participation. This study was conducted in line with the Declaration of Helsinki.

Experimental design

Baseline measurements were conducted under controlled conditions. Immediately before the race we measured body mass, heart rate and core body temperature. Subsequently, subjects walked 30 km (men: 37% / women: 31%), 40 km (men: 34% / women: 40%) or 50 km (men: 29% / women: 29%) at a self selected pace. During exercise, we assessed heart rate and core body temperature every 5 km, and all subjects registered their fluid intake using a diary. Immediately after finishing, all baseline measurements were repeated.

Table 1. Demographic characteristics and health status for men (n=56) and women (n=42)

	Men	Women	P-value
Demographic characteristics			
Age (yr)	61 ± 14	56 ± 15	0.92
Height (cm)	180 ± 7	165 ± 7	<0.001
Weight (kg)	83.9 ± 13.5	65.5 ± 10.3	<0.001
Body-mass index (kg/m ²)	26.0 ± 3.3	24.0 ± 3.2	0.004
Body fat (%)	25 ± 6	35 ± 5	<0.001
Waist circumference (cm)	96 ± 10	82 ± 9	<0.001
Health status			
Physical activity (hours/week)	3.1 ± 5.1	3.3 ± 3.4	0.86
≥5 times/week ≥30 min exercise (%)	79%	85%	0.48
Mean arterial pressure (mm Hg)	104 ± 11	104 ± 11	0.13
Use of prescribed medicines	23 (41%)	11 (26%)	0.13
Diuretics	6 (11%)	2 (5%)	0.29
Anti-hypertensive drugs	16 (29%)	3 (7%)	0.008
Statins	11 (20%)	3 (7%)	0.08
Other (e.g. painkillers, asthma, antirheumatics)	3 (5%)	5 (12%)	0.24
Pathology	35 (63%)	23 (55%)	0.44
Hypertension	14 (25%)	7 (17%)	0.32
Cardiovascular diseases	16 (29%)	7 (17%)	0.17
Hypercholesterolemia	16 (23%)	5 (12%)	0.15
Other (e.g. osteoporosis, skin disease, asthma)	21 (38%)	17 (41%)	0.77

P-value refers to an unpaired t-test or Chi-square test between men and women.

Measurements

Subject characteristics. At baseline, body mass (Seca 888 scale, Hamburg, Germany) and height were measured. Subsequently a four-point skinfold thickness

measurement (biceps, triceps, sub-scapular, supra-iliac) was obtained in order to calculate the lean body mass.¹⁴ Waist circumference was measured midway between the lower rib margin and iliac crest. Thereafter, resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min seated rest. Finally, all subjects completed a questionnaire about their physical activity and health status. Women were defined as postmenopausal when the last menstruation in absence of hormonal treatment occurred at least one year before the start of this experiment.

Fluid balance. Before the start of exercise, and directly after finishing, body mass was measured. The relative change in body mass (in %) between both measurements was calculated. Dehydration was defined as a body mass loss of 2% or more, which is a frequently applied definition for dehydration in field studies.^{13, 15} Furthermore, all subjects received written and individual oral instructions concerning the registration of their fluid intake. Subjects were allowed to drink *ad libitum*, while they registered the time and amount (standard sized cups, bottles, etc.) of their individual fluid intake from 12 hours preceding the start until the end of the experiment.

Blood analysis. After 5 minutes of rest in an upright position, two ml of venous blood was drawn at baseline and directly after finishing in order to determine plasma levels of sodium, hematocrit (Rapidpoint® 400, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.) and hemoglobin (HemoCue AB, Ängelholm, Sweden). Hyponatremia and hypernatremia were defined as a plasma sodium concentration of ≤ 135 mmol/L and ≥ 145 mmol/L, respectively.^{12, 16} Relative changes in plasma volume (%), were calculated from changes in blood hematocrit and haemoglobin concentrations according Dill and Costill's equation:¹⁷ $\Delta PV (\%) = 100 * ((Hb_{\text{baseline}} / Hb_{\text{post-exercise}}) * (1 - Hct_{\text{post-exercise}} * 0.01)) / (1 - Hct_{\text{baseline}} * 0.01) - 100$.

Urine specific gravity. Subjects were asked to provide a 5 mL urine sample at baseline as well as directly after finishing. The samples were immediately analyzed to determine the urine specific gravity (Clinitek Status® Analyzer; Siemens Healthcare Diagnostics, Tarrytown, USA).

Core body temperature. Core body temperature was determined using a portable telemetry system (CorTemp™ system, HQ Inc, Palmetto, USA), which has been demonstrated to be safe and reliable.^{18, 19} Participants ingested an individually calibrated telemetric temperature sensor the evening preceding the experiment, in order to avoid any interaction with fluid ingestion.²⁰ Prior to the start, core body temperature of each individual participant was measured using an external recorder. Baseline core body temperature was determined as the average of 3 consecutive measurements. Subsequently, core body temperature was similarly determined at every 5 km during the experiment. The highest value of these

measurements was presented as maximum core body temperature.

Exercise intensity. Heart rate was measured simultaneously with core body temperature (i.e. every 5 km point, 3 consecutive measurements), using a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland). Mean heart rate during exercise was calculated as the average heart rate, excluding the values derived directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate ($208 - 0.7 \cdot \text{age}$).²¹

Ambient conditions. Throughout the experiment, dry bulb, wet bulb, and globe temperatures were measured every 30 minutes using a portable climate monitoring device (Davis instruments inc., Hayward, U.S.A.) positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula: $\text{WBGT} = 0.1 (T_{\text{dry bulb}}) + 0.7 (T_{\text{wet bulb}}) + 0.2 (T_{\text{globe}})$.⁵

Statistics

All values are presented as means with standard deviation, unless indicated otherwise. Statistical analyses were performed using Statistical Package for Social Sciences 16.0 (SPSS, Chicago, Illinois). The level of statistical significance was set at $p < 0.05$. The normality of the data distribution was examined by the Kolmogorov-Smirnov test. To compare differences between men and women or pre- and postmenopausal women, an unpaired *t*-test was used for continuous variables. To assess differences in the incidence of hypernatremia and high urine specific gravities between the groups, a Pearson's Chi-square test was applied. A multivariable logistic regression analyses was used to model the relation between sex and the incidence of dehydration as indicated by $\geq 2\%$ body mass loss. Crude Odds Ratios (crOR) were obtained by entering the incidence of $\geq 2\%$ body mass loss as dependent variable and sex (categorized) as independent variable. Differences in anthropometrical data, health status, exercise characteristics and hydration status, between men and women were tested for their potential confounding effects on the incidence of dehydration as indicated by $\geq 2\%$ body mass loss. To evaluate the confounding effect size of these covariates we added one factor at a time to our regression model based on sex. When the Odds Ratio changed with more than 10% compared to the crOR after introducing a potential confounder, the confounding effect was considered large enough and therefore we retained this factor in our final model for the estimation of the incidence of dehydration based on sex (adjusted OR). All Odds Ratios are presented with their 95% confidence intervals (CIs). To identify factors that contribute to the development of $\geq 2\%$ body mass loss, a backward stepwise linear regression analysis was used. We have included sex, age, BMI, exercise duration, exercise intensity and fluid intake as potential determinants of the presence of dehydration. To exclude the possibility that baseline differences in body mass between men and women may impact the presence $\geq 2\%$ body mass

loss, we additionally performed a Pearson correlation and a backward logistic regression analysis to study the contribution of body mass to our outcomes. . A two-way repeated measures analysis of variance was used to test the changes in plasma sodium concentration over time between men and women.

Results

One male subject withdrew from participation after baseline measurements and was therefore excluded from analysis. We found no sex difference regarding age,

Table 2. Exercise characteristics and hydration status for men (n=56) and women (n=42).

	Men	Women	P-value
Exercise characteristics			
Exercise duration (hh:mm)	8:15 ± 1:39	8:54 ± 1:38	0.054
Speed (km/h)	4.8 ± 0.6	4.5 ± 0.7	0.043
Baseline T_c (°C)	37.2 ± 0.4	37.4 ± 0.4	0.025
Maximum T_c (°C)	38.1 ± 0.3	38.3 ± 0.4	0.018
Increase in T_c (°C)	1.0 ± 0.4	0.9 ± 0.4	0.59
Exercise intensity (%)	68 ± 11	70 ± 10	0.28
Hydration status			
Fluid intake (L)	2.1 ± 1.2	2.2 ± 0.8	0.80
Water (%)	57%	52%	
Sports drink (%)	11%	16%	
Other (%)	32%	32%	
Fluid intake / hour / kg (mL)	2.9 ± 1.1	3.7 ± 1.3	0.002
Body mass change (kg)	-1.4 ± 0.9	-0.6 ± 0.5	<0.001
Body mass change (%)	-1.6 ± 1.0	-0.9 ± 0.8	<0.001
Dehydration; >2% body mass loss (%)	19 (34%)	5 (12%)	0.01
Calculated plasma volume change (%)	-1.5 ± 9.0	1.4 ± 7.4	0.09
Baseline urine specific gravity ≥1.030 g/mL	15 (27%)	14 (33%)	0.48
Finish urine specific gravity ≥1.030 g/mL	25 (45%)	11 (26%)	0.063
Baseline sodium concentration (mmol/L)	141.7 ± 2.0	141.3 ± 1.4	0.24
Finish sodium concentration (mmol/L)	143.2 ± 2.6	140.9 ± 1.9	<0.001
Baseline hypernatremia (%)	2 (4%)	0 (0%)	0.22
Finish hypernatremia (%)	15 (27%)	0 (0%)	<0.001

P-value refers to an unpaired t-test or Chi-square test between men and women. T_c , core body temperature.

physical activity level, mean arterial pressure and the presence of hypertension, cardiovascular diseases and hypercholesterolemia (Table 1). Apart from anti-hypertensive drugs, no difference in the use of medication between both groups was present (Table 1). Men showed a higher BMI, height, weight and waist circumference, but lower fat percentage compared with women (Table 1). To correct for the anthropometric differences between men and women, BMI and the use of anti-hypertensive drugs were included in the multivariate logistic regression analysis.

Exercise characteristics

Relative humidity, dry bulb and wet bulb temperature were 67%, 14.2°C and 12.2°C, respectively, in the early morning and 78%, 20.5°C and 16.7°C,

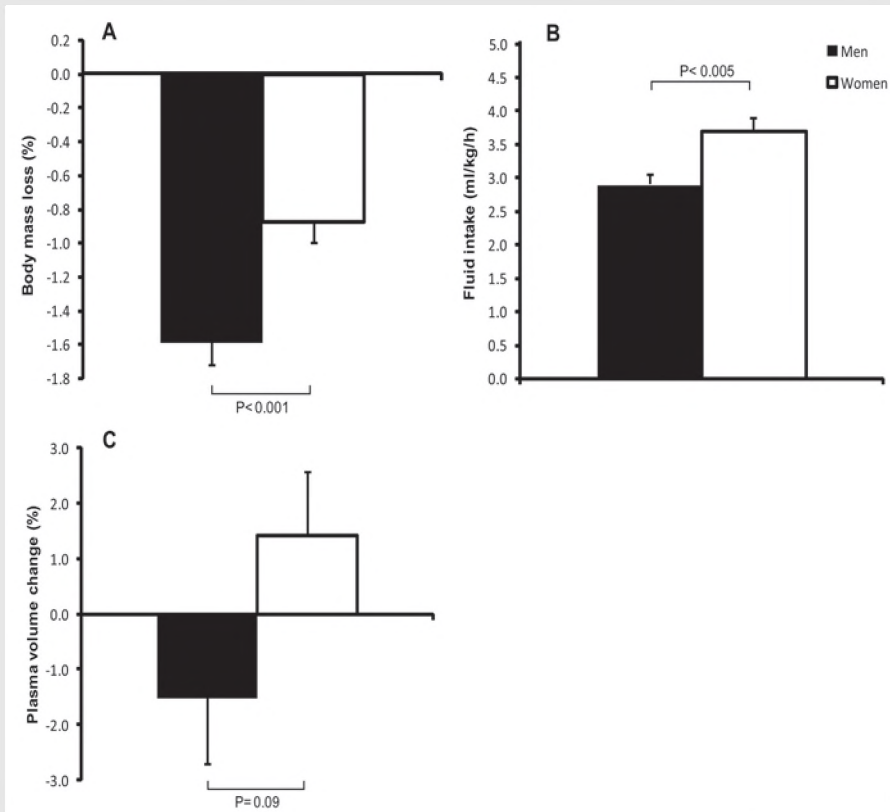
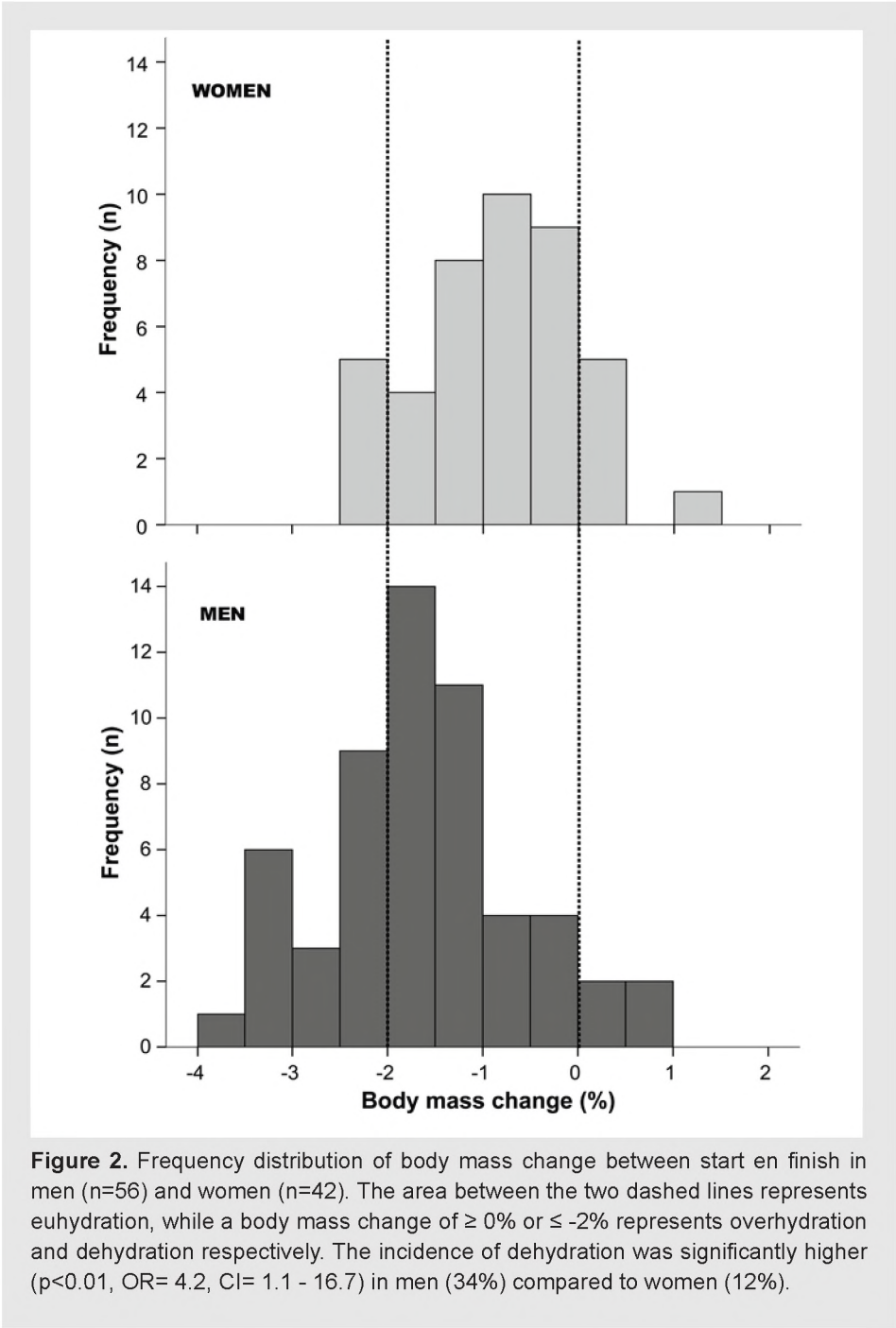


Figure 1. Fluid balance parameters in men (n=56) and women (n=42). Men demonstrated a significantly larger body weight loss (A) and higher fluid intake (B) compared to women, whilst also a trend in plasma volume changes (C) amongst both groups was observed. Data is presented as mean \pm SE.



respectively, in the afternoon. WBGT during the experiment varied between 13.2°C and 20.6°C. Duration of the exercise bout was comparable between both groups (Table 2). Subjects performed exercise at $69 \pm 11\%$ of their predicted maximal heart rate, which was similar between men and women (Table 2). Whilst baseline and maximal core body temperature were significantly higher in women than in men, the increase in core body temperature was comparable between both groups (Table 2).

Body mass losses

A significant decrease in body mass was observed in both groups after finishing, which was significantly larger in men than in women (Figure 1). Whilst 25% of our population demonstrated $\geq 2\%$ body mass loss, men more often exceeded this threshold than women (34% *versus* 12%, respectively, $\text{crOR}=3.8$, $\text{CI}=1.3\text{--}11.3$, Figure 2). After correction for BMI, anti-hypertensive drugs, speed, maximum core body temperature and fluid intake, the relation between sex and $\geq 2\%$ body mass loss remained significant (adjusted $\text{OR}=4.2$, $\text{CI}=1.1\text{--}16.7$). Indeed, a backward logistic regression analysis revealed that only sex ($\text{OR}=2.7$, $\text{CI}=0.9\text{--}8.5$) and fluid intake ($\text{OR}=0.6$, $\text{CI}=0.4\text{--}0.9$) significantly contributed to the presence of $\geq 2\%$ body mass loss. In addition, we found no correlation between baseline body mass and body mass changes after prolonged exercise within men ($p=0.79$, $r=-0.04$) and women ($p=0.86$, $r=0.03$). This suggests that differences in body mass between men and women do not explain the sex differences in the presence of $\geq 2\%$ body mass loss.

Table 3. Hydration status in pre-menopausal ($n=12$) and post-menopausal ($n=30$) women during prolonged walking.

	Pre-menopausal	Post-menopausal	P-value
Hydration status			
Fluid intake (L)	2.2 ± 0.9	2.2 ± 0.8	0.79
Fluid intake / hour / kg (mL)	3.4 ± 1.5	3.8 ± 1.2	0.42
Body mass change (kg)	-0.4 ± 0.6	-0.7 ± 0.5	0.25
Body mass change (%)	-0.6 ± 0.8	-1.0 ± 0.8	0.18
Dehydration (%)	0 (0%)	5 (17%)	-*
Calculated plasma volume change (%)	-0.2 ± 6.3	2.0 ± 7.9	0.39
Urine specific gravity ≥ 1.030 g/mL	1 (8%)	10 (33%)	0.096
Baseline sodium concentration (mmol/L)	140.8 ± 1.0	141.5 ± 1.5	0.13
Finish sodium concentration (mmol/L)	139.9 ± 1.8	141.2 ± 1.8	0.036
Hypernatremia (%)	0 (0%)	0 (0%)	-*

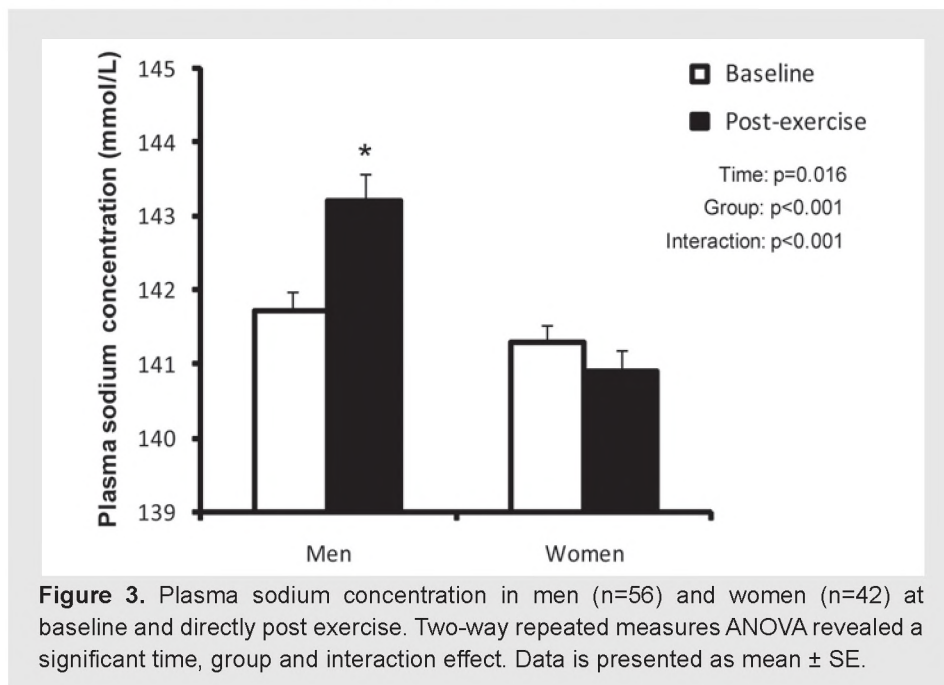
P-value refers to an unpaired t-test or Chi-square test between pre-menopausal and post-menopausal women. * The p-value could not be calculated as (one of) the groups had a incidence of 0%

Fluid balance

The average fluid intake preceding the start of the experiment was 1.2 ± 0.6 liter in men and 1.0 ± 0.6 liter in women and did not statistically differ between the groups ($P=0.21$). Also, the total amount and type of fluid intake during exercise was not different between groups (Table 2). However, when corrected for body mass and exercise duration, men demonstrated a significantly lower fluid intake during exercise than women (Figure 1). In addition, men showed a decrease in post exercise plasma volume (-1.5%), whilst women demonstrate an increase (1.4% , Figure 1). Moreover, men demonstrated a higher incidence of post-exercise urine specific gravity levels ≥ 1.030 g/mL compared to women (Table 2). Comparison between premenopausal and postmenopausal women revealed no differences for fluid intake, body mass changes or urine specific gravity levels (Table 3).

Sodium levels

Whilst men demonstrated an increase in plasma sodium levels, a decrease was observed in women (Figure 3), leading to higher post-exercise blood sodium levels in men (Table 2). In addition, the incidence of hyponatremia (sodium level ≥ 145 mmol/L) was significantly higher in men compared to women (27 *versus* 0%, respectively, $P<0.001$, Figure 4). The exercise-induced change in plasma sodium levels was not different between premenopausal (-0.9 ± 1.9 mmol/L) and postmenopausal women (-0.3 ± 2.0 mmol/L, *t*-test; $P=0.41$).



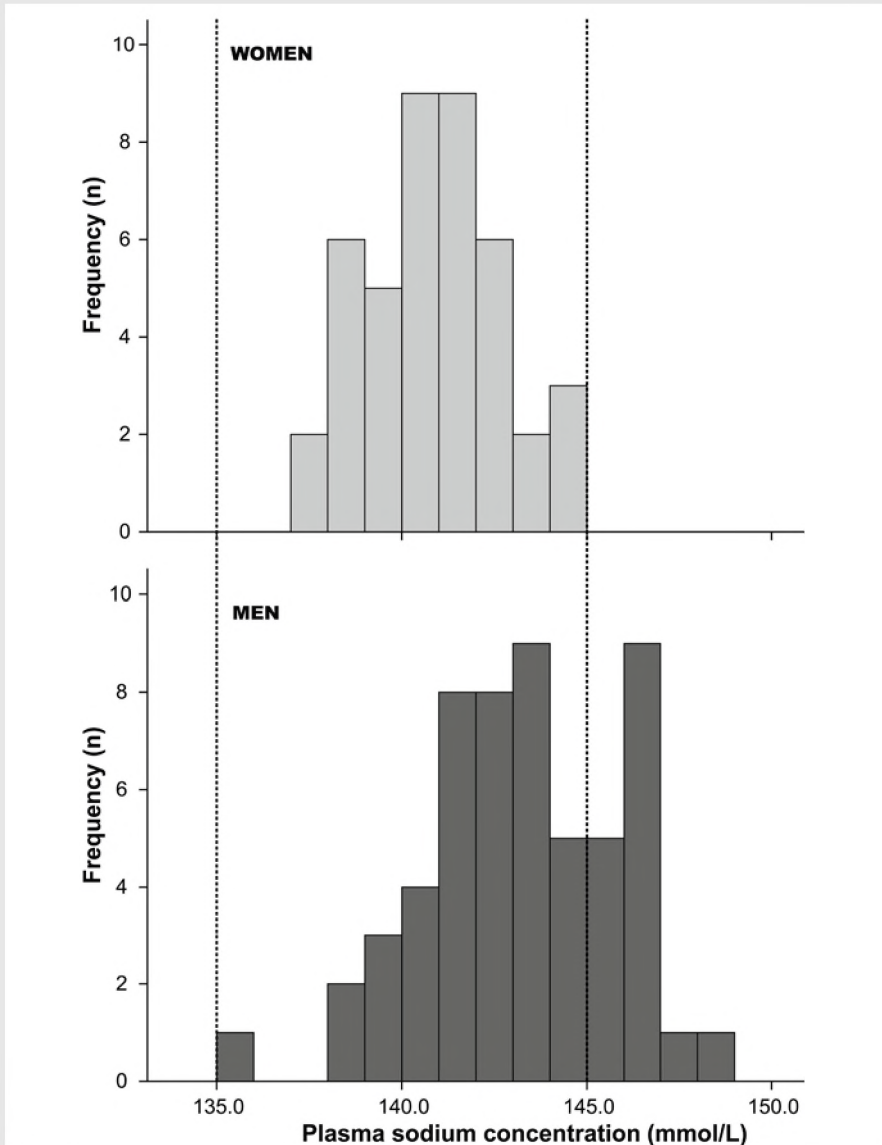


Figure 4. Frequency distribution of the changes in plasma sodium concentration in men ($n=56$) and women ($n=42$) after prolonged moderate intensity exercise. The dashed line represents no change in plasma sodium concentration, meaning that baseline and post-exercise plasma sodium levels were similar. This figure also illustrates the sex difference: i.e. men demonstrate an increase in plasma sodium levels, whilst women show a slight decrease ($p<0.001$).

Discussion

This study examined the impact of sex on fluid balance responses during prolonged exercise in a large group of men and women. A clear difference between the sexes was observed, with a significantly lower fluid intake and larger loss of body mass in men. Moreover, a sex difference is further supported by the larger increase in plasma sodium levels and higher incidence of hyponatremia in men. Although none of our subjects reported clinical signs or health problems, the findings of this study indicate that important differences are present in the hydration status between men and women during prolonged exercise.

Given the prolonged duration (8h30min) and intensity (69% of their maximum predicted heart rate) of the exercise bout, our subjects are susceptible to disturbances in their fluid balance.^{6, 13} Whilst the relation between fluid disturbances and the duration or intensity of exercise is widely acknowledged,^{6, 7, 22, 23} we focused on dehydration after prolonged moderate-intensity exercise. In our study, the average body mass loss was 1.6% in men and 0.9% in women. The magnitude of this response is in line with a previous study that used models to predict body mass changes after prolonged running.²⁴ Moreover we found that 25% of the participants lost more than 2% of their body mass, which is frequently used as a marker for dehydration. It is important to notice that this high incidence was observed during mild to moderate ambient conditions, and *ergo* is likely to increase when the same type of exercise is performed under more strenuous ambient conditions.

The principle finding of our study is that men demonstrated a larger body mass loss compared to women during prolonged exercise. Decreases in body mass after exercise relates, at least partly, to water loss.²⁵ Therefore, men might be more prone to develop dehydration during exercise than women. This observation raises the question what the potential mechanisms might be that contributes to this sex difference. Although longer duration and high-intensity exercise predisposes subjects to develop dehydration,^{6, 26, 27} men and women performed exercise at a similar relative intensity and exercise duration in our study. Another potential explanation relates to the amount and type of fluid intake during exercise. Although there was no difference in the *type* of fluid intake (e.g. water, sports drink or other), men demonstrated a 22% lower rate of fluid intake than women (2.9 *versus* 3.7 ml/hour/kg). This observation is in parallel with a previous laboratory study.²⁸ Alternatively, sex differences may influence the thirst stimulus in the hypothalamus. Since advanced age attenuates the thirst stimulus,^{27, 29} we matched age between the various groups in our study. Moreover, increased core body temperature influences the perception of thirst.³⁰ Interestingly, women demonstrated a slightly, but significantly higher maximum core body temperature during exercise than men (38.3°C *versus* 38.1°C), which may partially contribute to their higher fluid intake. Nonetheless, even after correction for fluid intake in

our statistical model, men demonstrated a higher risk for dehydration compared to women. This makes it unlikely that fluid intake alone explains the sex difference in the occurrence of dehydration.

Combining fluid intake with the absolute body mass change, we were able to roughly estimate the fluid loss.²² Men demonstrated a significantly greater rate of fluid loss than women during exercise (424 ± 162 ml/h *versus* 311 ± 93 ml/h, $P < 0.001$). Although this approach does not correct for respiratory and gastrointestinal fluid losses, previous studies showed that metabolic water production in the muscles compensates the respiratory fluid losses,^{31, 32} whilst gastrointestinal losses are normally negligible (i.e. 100 mL/day).³³ Therefore, fluid loss during exercise is assumed to be predominantly attributed to sweating,^{10, 13} and subordinately to urinary excretion. A greater sweating rate may contribute to the larger fluid loss in men. Indeed, previous studies found higher sweating rates in men compared to women.^{8, 9} While these sex differences in sweating response have been attributed to anthropometric differences (e.g. body composition, sweat gland density and sweat gland threshold),³⁴⁻³⁷ a recent study also demonstrated that women have a lower sweat gland cholinergic sensitivity compared to men,³⁸ which may contribute to our findings. The higher incidence of dehydration in men may, at least partly, be explained by their larger fluid loss, which was not compensated by additional fluid intake.

The antidiuretic hormone arginine vasopressin (AVP) regulates fluid balance by increasing renal water reabsorption and thereby reducing urinary output (and thus preventing dehydration). Sex hormones modulate the synthesis and osmotic regulation of AVP.³⁹⁻⁴¹ At rest, men have a higher baseline plasma AVP level and a greater sensitivity to changes in plasma osmolality than women.^{42, 43} Nonetheless, no differences in urinary output between men and women were found after osmotic stimulation.⁴³ Also exercise represents a stimulus that induces a change in AVP levels to maintain fluid balance.⁴⁴ Possibly, an attenuated exercise-induced increase in AVP in men may contribute to our findings.

Our findings between men and women may relate to the menstrual cycle, especially since the menstrual cycle alters body fluid regulation.⁴⁵ In our study, ~70% of the female population was postmenopausal. Nonetheless, fluid intake and changes in body mass, plasma volume and plasma sodium were not different between pre- and postmenopausal women. Therefore, the sex-related differences in our study unlikely relate to the menstrual cycle.

Limitations. Dehydration in our study was assessed by body mass changes before and after exercise. Although this approach is a frequently and popular method, especially during field studies,^{6, 46-48} some limitations must be considered. A recent study showed that using body mass changes in men and women is a reliable and accurate method to assess total body water changes after prolonged

running.²⁵ Nonetheless, this measure does not account for substrate oxidation and metabolic water production during prolonged exercise.^{49, 50} Consequently, body mass loss during exercise is not solely due to fluid loss, as also discussed in response to the findings of Baker *et al.*^{51, 52} Therefore, one may question the validity of the subjective 2% cut off value for the definition of dehydration. Nonetheless, we believe that our methods are valid to examine the primary aim of the study, especially since we also included other markers of hydration (i.e. sodium concentration and plasma volume changes) that reinforce the sex differences in hydration status.

Perspectives

We found that men demonstrated larger decreases in body mass and a higher incidence of dehydration after prolonged exercise than women. This difference was reinforced by backward linear regression analysis that revealed that sex and fluid intake were the only parameters that relate to $\geq 2\%$ body mass loss during prolonged walking. These findings suggest that the control of the fluid balance is regulated differently in men and women during exercise. Although sex differences in fluid intake and sweat rate can partially explain our results, these parameters do not fully account for the observed sex differences. Our findings suggest that sex must be considered when providing fluid replacement advices and/or guidelines.

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Click to - and produce > 100 Pa

Total body fluid (42 L)

Intracellular

Interstitium 10.0 L

Extracellular

Interstitium 10.0 L

Plasma 3.0 L

Cell body temperature

Distance
Grav
Interstitium



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Chapter 4

The impact of obesity on physiological responses during prolonged exercise

Thijs M.H. Eijssvogels
Matthijs T.W. Veltmeijer
Tim H.A. Schreuder
Fleur Poelkens
Dick H.J. Thijssen
Maria T.E. Hopman

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Abstract

Background: Prolonged, moderate-intensity exercise training is routinely prescribed to subjects with obesity. In the general population, this type of exercise can lead to fluid and sodium imbalance. However, little is known whether obesity alters the risk of fluid and sodium imbalances.

Objective: This study examined physiological responses, such as core body temperature, fluid and sodium balance, in lean ($BMI < 25$), overweight ($25 < BMI < 30$) and obese ($BMI > 30$) subjects during prolonged moderate-intensity exercise.

Subjects: 93 volunteers (24-80 years), stratified for BMI, participated in the Nijmegen Four Days Marches and walked 30-50 km at a self-selected pace. Heart rate and core body temperature were recorded every 5 km. Subjects reported fluid intake, whilst urine output was measured and sweat rate was calculated. Baseline and post-exercise plasma sodium levels were determined, and urinary specific gravity levels were assessed before and after exercise.

Results: BMI-groups did not differ in training status preceding the experiment. Exercise duration ($8h41min \pm 1h36min$) and intensity ($72 \pm 9\% HR_{max}$) were comparable across groups, whilst obese subjects tended to have a higher maximum core body temperature than lean controls ($p=0.06$). Obese subjects demonstrated a significantly higher fluid intake ($p<0.001$) and sweat rate ($p<0.001$), but lower urine output ($p<0.05$) compared to lean subjects. In addition, higher urine specific gravity levels were observed in obese versus lean subjects after exercise ($p<0.05$). Furthermore, plasma-sodium concentration did not change in lean subjects after exercise, whilst plasma sodium levels increased significantly ($P<0.001$) in overweight and obese subjects. Also, overweight and obese subjects demonstrated a significantly larger decrease in body mass after exercise than lean controls ($p<0.05$).

Conclusion: Obese subjects demonstrate a larger deviation in markers of fluid and sodium balance than their lean counterparts during prolonged moderate-intensity exercise. These findings suggest that overweight and obese subjects, especially under strenuous environmental conditions, have an increased risk to develop fluid and sodium imbalances.

Introduction

Obesity is a rapidly growing problem in western countries. Obesity is associated with a markedly increased risk to develop chronic diseases later in life, such as type 2 diabetes, cardiovascular diseases and cancer.¹⁻⁶ An inactive lifestyle plays a dominant role in the development of obesity and its related health problems.⁷⁻⁹ Indeed, physically active obese subjects have a reduced risk to develop diabetes mellitus, and report better fasting glucose levels and cardiovascular risk profiles compared to their physically inactive peers.¹⁰⁻¹³ Therefore, exercise training is routinely prescribed to individuals with obesity,¹⁴⁻¹⁶ with a preference for moderate-intensity exercise that can be performed for prolonged periods (e.g. brisk walking). Although the health benefits are well described, little is known about the physiologic responses during endurance exercise in obese subjects.

Prolonged exercise is associated with significant fluid loss and an increase in core body temperature.¹⁷ These exercise induced physiological changes may result in the development of hyperthermia, dehydration and sodium imbalance, consequently followed by impaired aerobic exercise performance levels and potential health problems.¹⁷⁻²¹ Obesity may be related to altered physiological responses to exercise. For example, due to the larger body surface area and a greater number of sweat glands, obese subjects typically have larger fluid losses.²² Secondly, obese subjects demonstrate altered thermoregulatory response.²³ Previous studies showed that body mass index (BMI) was related to the occurrence of heat disorders in highly trained, young soldiers.^{24, 25} It is, however, unknown whether obesity in the general population is related to the development of fluid and sodium imbalances during prolonged exercise.

Therefore the purpose of this study was to assess whether physiological responses to prolonged moderate-intensity exercise in obese subjects differ from overweight and lean subjects. For this purpose, we examined exercise intensity, core body temperature, fluid and sodium parameters in a large, heterogeneous group of subjects before, during and immediately after prolonged exercise. We hypothesized that obese subjects demonstrate a larger fluid loss and a higher core body temperature compared to lean peers, which might result in a higher incidence of dehydration and hypernatremia. This study is of particular clinical interest since prolonged, moderate-intensity exercise is typically prescribed to patients with obesity.

Methods

Subjects

Ninety-three participants of the Nijmegen Four Days Marches (an annual four day walking event in The Netherlands) were included and stratified for body

mass index (BMI). Subjects were defined as lean (BMI <25), overweight (BMI 25 – 30) or obese (BMI >30 kg/m²) (Table 1). Data were collected during the first day of this walking march only. A written informed consent was obtained from all participants prior to the start of the study. This study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Centre, and was conducted in accordance with the Declaration of Helsinki.

Experimental design

At baseline (one or two days before the exercise bout), subject characteristics and body composition were measured under controlled conditions (Figure 1). Moreover, fluid balance was examined by a blood and urine sample. Immediately before the start of exercise, fluid balance and core body temperature were examined. Thereafter, subjects participated in the first day of the Nijmegen Four Days Marches and walked 30 km (31%), 40 km (47%) or 50 km (22%). During exercise, fluid balance, core body temperature and exercise intensity were measured every subsequent 5 km. Immediately after finishing, exercise intensity and core body temperature were measured again, whilst fluid balance was re-assessed (Figure 1).

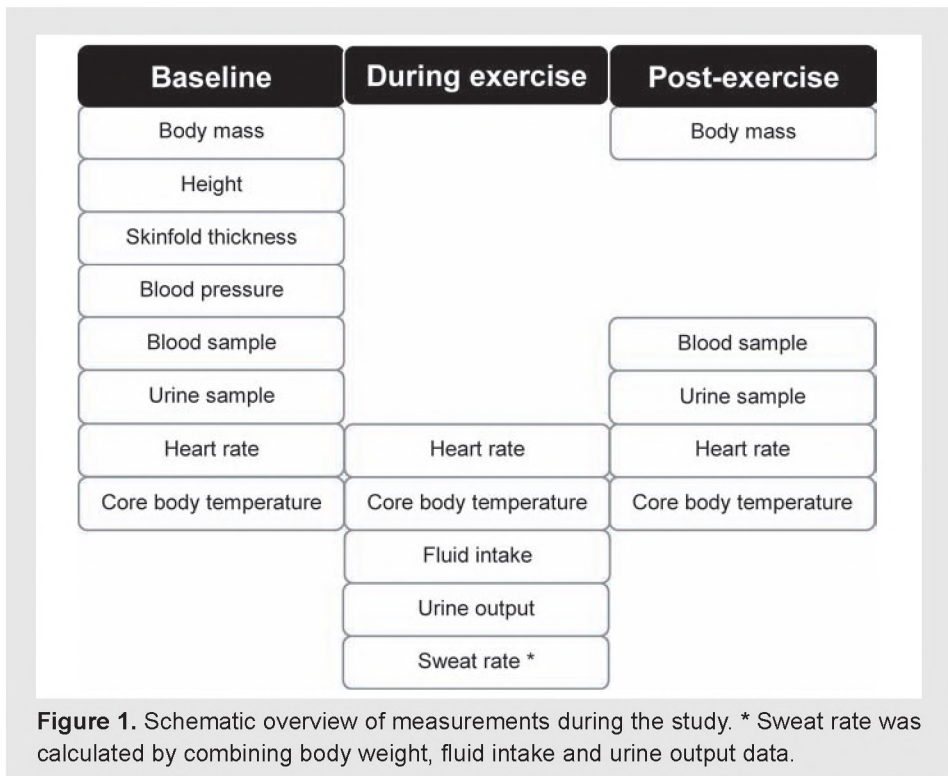


Table 1. Subject characteristics and details about physical activity and the presence of prescribed medicines and pathology presented per BMI group.

	BMI < 25	BMI 25 - 30	BMI > 30	P-value
Demographic characteristics				
Gender (men:women)	15:16	15:16	18:13	-
Age (yr)	56 ± 19	56 ± 11	53 ± 9	0.61
Height (cm)	174 ± 11	174 ± 9	174 ± 9	0.96
Weight (kg)	69.6 ± 11.0	83.0 ± 8.7 ¹	100.4 ± 10.9 _{1,2}	<0.001
Body-mass index (kg/m ²)	22.8 ± 1.9	27.4 ± 1.4 ¹	33.0 ± 2.0 ^{1,2}	<0.001
Body fat (%)	29 ± 6	35 ± 6 ¹	39 ± 5 ^{1,2}	<0.001
Lean body mass (kg)	49.4 ± 10.6	54.4 ± 8.7	61.8 ± 10.4 ^{1,2}	<0.001
Body Surface Area (m ²)	1.83 ± 0.20	1.98 ± 0.16 ¹	2.15 ± 0.17 ^{1,2}	<0.001
Abdominal circumference (cm)	85 ± 8	96 ± 6 ¹	110 ± 9 ^{1,2}	<0.001
Waist circumference (cm)	93 ± 6	102 ± 6 ¹	111 ± 7 ^{1,2}	<0.001
Waist to hip ratio	0.91 ± 0.07	0.94 ± 0.06	0.99 ± 0.08 ^{1,2}	<0.001
Health status				
Physical activity				
Sports (hours/week)	3.8 ± 4.2	2.1 ± 2.7	2.9 ± 4.4	0.61
Training status (km/year) *	568 ± 642	467 ± 693	577 ± 577	0.54
Mean arterial pressure (mm Hg)	98 ± 11	104 ± 11	106 ± 12 ¹	0.021
Use of prescribed medicines	14 (45%)	20 (65%)	25 (81%) ¹	-
Diuretics	1 (3%)	1 (3%)	4 (13%)	-
Anti-hypertensive drugs	4 (13%)	8 (26%)	10 (32%)	-
Statins	3 (10%)	3 (10%)	5 (16%)	-
Pathology	17 (55%)	22 (71%)	20 (65%)	-
Hypertension	6 (19%)	8 (26%)	10 (32%)	-
Hypercholesterolemia	4 (13%)	4 (13%)	7 (23%)	-
Skin disease	2 (7%)	7 (23%)	5 (16%)	-
Osteoporosis	3 (10%)	3 (10%)	2 (7%)	-
Asthma	1 (3%)	4 (13%)	5 (16%)	-
Diabetes	1 (3%)	0 (0%)	4 (13%)	-

^{1,2} = Post-hoc significant difference in relation to BMI <25 and BMI 25 – 30 groups respectively. *Ln-transformation was applied as a non-Gaussian distribution was present.

Measurements

Subject characteristics. Body mass was measured using a Seca 888 scale

(Hamburg, Germany). Body surface area was calculated according the formula of Dubois *et al.*²⁶ A four-point skinfold thickness measurement (biceps, triceps, sub-scapular, supra-iliac) was obtained in order to calculate the lean body mass.²⁷ Waist circumference was measured midway between the lower rib margin and iliac crest. Hip circumference was measured at the level of widest circumference over greater trochanters. Waist to-hip ratio was calculated as waist circumference divided by hip circumference. Resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min seated rest. Finally, all subjects completed a questionnaire about their physical activity (hours of sport participation per week), training status (walking-specific training history in the year preceding the walking march) and health status (pathology and use of medication).

Fluid balance. The relative change in body mass (in %) between the measurement immediately before the start and directly after finishing was calculated and dehydration was defined as a body mass loss of 2% or more.^{17, 28} Furthermore, all subjects received written and individual oral instructions concerning the registration of their fluid intake. During exercise, subjects were allowed to drink ad libitum, whereas they registered the time (in blocks of 1 hour), amount (using standard sized cups and bottles) and type ('water', 'sports drink' or 'other') of their individual fluid intake in a diary.

Urine analysis. The 5 mL urine sample that was provided by all subjects was immediately analyzed to determine urine specific gravity (Clinitek Status® Analyzer; Siemens Healthcare Diagnostics, Tarrytown, USA). Values of ≥ 1.030 g/mL indicate dehydration.^{21, 29} To determine the amount of urine output, subjects were instructed to exclusively urinate into a specialized collecting bag (Roadbag/Ladybag; KETs GmbH, Köln, Germany). Bags were collected and weighed at the laboratory within 0.1 g accuracy (PT 1500; Sartorius AG, Göttingen, Germany).

Sweat rate. Sweat rate (mL/h) was calculated by combining body weight, fluid intake and urine output data using the formula: $\text{Sweat rate (mL/h)} = (\text{pre-exercise body weight} - \text{post-exercise body weight} + \text{fluid intake} - \text{urine output}) / \text{exercise duration}$.²¹

Blood analysis. Two ml of venous blood were drawn in order to determine plasma levels of sodium, hematocrit and hemoglobin (Rapidpoint® 400, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.). Hyponatremia and hypematremia were defined as a plasma sodium concentration of ≤ 135 mmol/L and ≥ 145 mmol/L, respectively.^{30, 31} Relative changes in plasma volume (%), were calculated from changes in blood hematocrit and haemoglobin concentrations according Dill and Costill's equation.³²

Core body temperature. Core body temperature was determined using a portable telemetry system (CorTemp™ system, HQ Inc, Palmetto, USA), which has been demonstrated to be safe and reliable.^{33, 34} Participants ingested an individually calibrated telemetric temperature sensor the evening preceding the experiment. Core body temperature was measured using an external recorder and determined as the average of 3 consecutive measurements on each occasion. The highest value of the core body temperature during the exercise bout was presented as peak core body temperature.

Exercise intensity. Heart rate was measured simultaneously with core body temperature (i.e. 3 consecutive measurements), using a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland). Mean heart rate during exercise was calculated as the average heart rate, excluding the values measured directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate $(208 - 0.7 * \text{age})$.³⁵

Ambient conditions. Throughout the experiment, dry bulb, wet bulb, and globe temperatures were measured every 30 minutes using a portable climate monitoring device (Davis instruments inc., Hayward, U.S.A.) positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated to gauge the heat risk, using the formula: $WBGT = 0.1 * (T_{\text{dry bulb}}) + 0.7 * (T_{\text{wet bulb}}) + 0.2 * (T_{\text{globe}})$.³

Statistical analysis

All values were presented as mean \pm standard deviation, unless indicated otherwise. Statistical analyses were performed using SPSS 16.0. The level of statistical significance was set at $p < 0.05$. The normality of the data distribution was examined by the Kolmogorov-Smirnov test. When data demonstrated a non-Gaussian distribution, Ln-transformation was applied. Comparisons between groups were assessed using One-way ANOVA for continuously distributed data and a post-hoc test with a Bonferroni correction for multiple comparisons was performed in case of a statistically significant difference. To assess whether the three groups demonstrated a different time course of core body temperature, we performed a linear mixed model analysis. All other parameters that may demonstrate group differences over time were assessed using a two-way repeated measures ANOVA. A binary logistic regression analysis was used to model the relation between BMI-groups and binominal distributed data. Odds Ratios were computed for the overweight ($OR_{\text{overweight}}$) and obese subjects (OR_{obese}), with the lean subjects (BMI < 25) serving as a reference group. All Odds Ratios are presented with their 95% confidence intervals (CI).

Results

The three BMI groups were not different in age and height, but did demonstrate differences in body composition (Table 1). Physical activity level and the training status preceding the walking march were comparable between lean, overweight and obese subjects (Table 1). Also, no differences in the prevalence of cardiovascular diseases, diabetes or other diseases were reported (Table 1). Obese subjects, however, had a higher mean arterial blood pressure and used more prescribed medication than their lean counterparts (Table 1).

Table 2. Exercise characteristics and fluid balance presented per BMI group. P-value refers to a One-Way ANOVA.

	BMI < 25	BMI 25 - 30	BMI > 30	P-value
Exercise characteristics				
Walking distance				
30 km	32%	36%	26%	
40 km	42%	45%	55%	
50 km	26%	19%	19%	
Exercise duration (hh:mm)	8:34 ± 1:57	8:44 ± 1:25	8:46 ± 1:26	0.89
Speed (km/h)	4.8 ± 0.7	4.6 ± 0.8	4.7 ± 0.6	0.43
Baseline T _c (°C)	37.5 ± 0.4	37.6 ± 0.5	37.5 ± 0.3	0.58
Maximum T _c (°C)	38.3 ± 0.3	38.4 ± 0.3	38.5 ± 0.3	0.06
Exercise intensity (%)	71 ± 10	71 ± 10	75 ± 8	0.16
Fluid balance				
Fluid intake (mL/hour)	239 ± 80	297 ± 104	366 ± 129 ^{1,2}	<0.001
Water (%)	61 ± 24	69 ± 21	62 ± 20	0.33
Sports drink (%)	10 ± 13	8 ± 12	18 ± 18 ²	0.018
Other (%)	29 ± 21	23 ± 19	20 ± 14	0.17
Sweat rate (mL/hour)	258 ± 89	395 ± 117 ¹	498 ± 169 ^{1,2}	<0.001
Urine output (mL/hour)*	75 ± 44	72 ± 59	44 ± 42 ¹	0.029
Urine specific gravity ≥1.030 g/mL	11 (36%)	16 (52%)	19 (61%) ¹	
Calculated plasma volume change (%)	3 ± 11	4 ± 15	-6 ± 9 ^{1,2}	0.003
Body mass change (kg)	-0.9 ± 0.8	-1.4 ± 0.8	-1.3 ± 1.0	0.049
Body mass change (%)	-1.2 ± 1.0	-1.6 ± 1.0	-1.2 ± 0.9	0.15
Dehydration; ≥2% body mass loss	6 (20%)	8 (27%)	5 (16%)	

^{1,2} = Post-hoc significant difference in relation to BMI <25 and BMI 25 – 30 groups respectively. *Ln-transformation was applied as a non-Gaussian distribution was present.

Exercise characteristics

All subjects successfully completed the exercise bout. The WBGT increased from 14.0 °C in the morning to a maximum of 25.0 °C in the afternoon. Walking distance, walking duration and walking speed did not significantly differ across groups (Table 2). Baseline core body temperature did not differ across groups (Table 2). Lean, overweight and obese subjects demonstrated a significant increase in core body temperature during exercise (Figure 2). However, the magnitude of this increase was significantly smaller in lean than in obese subjects ($p < 0.001$), and showed a trend for a smaller increase in lean compared to overweight subjects ($p = 0.073$). Also, the peak core body temperature tended to be higher in obese subjects compared to lean controls (Table 2). The exercise intensity was comparable between lean, overweight and obese subjects (respectively 71%, 71% and 75% of HR_{max}) (Table 2).

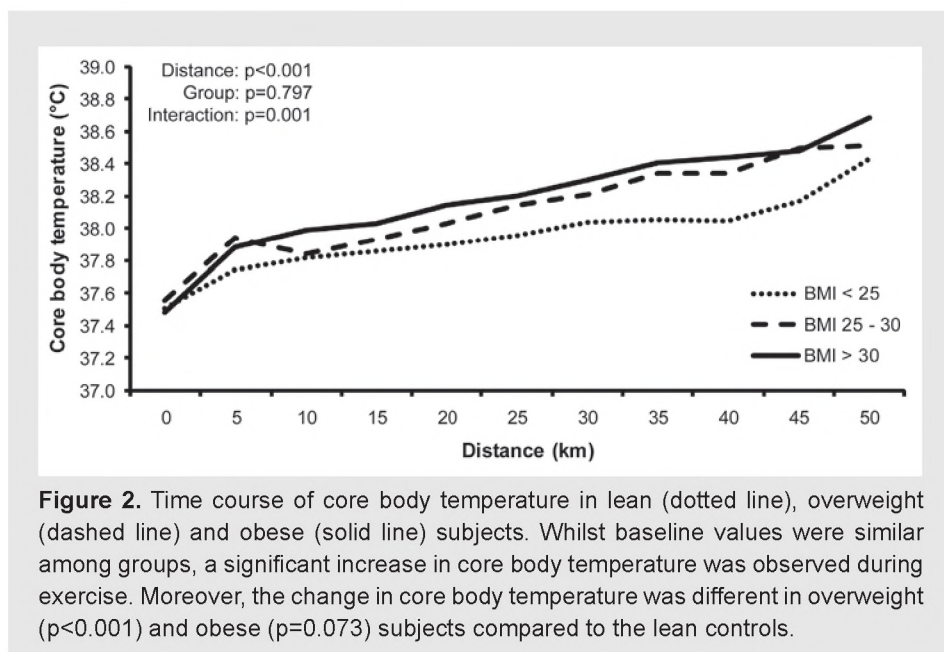


Figure 2. Time course of core body temperature in lean (dotted line), overweight (dashed line) and obese (solid line) subjects. Whilst baseline values were similar among groups, a significant increase in core body temperature was observed during exercise. Moreover, the change in core body temperature was different in overweight ($p < 0.001$) and obese ($p = 0.073$) subjects compared to the lean controls.

Fluid balance

Obese subjects reported a significantly greater fluid intake than lean subjects during exercise (Figure 3). Moreover, specification of fluid intake revealed that obese subjects had a slight but significant higher relative intake of sports drinks compared to the other BMI groups (Table 2). Overweight and obese subjects demonstrated also a significantly higher sweat rate compared to their lean counterparts (Figure 3). Although sweat rate was significantly related to body surface area ($p < 0.001$, $r^2 = 0.45$, Figure 4), overweight (200 ± 58 mL/h/m²) and

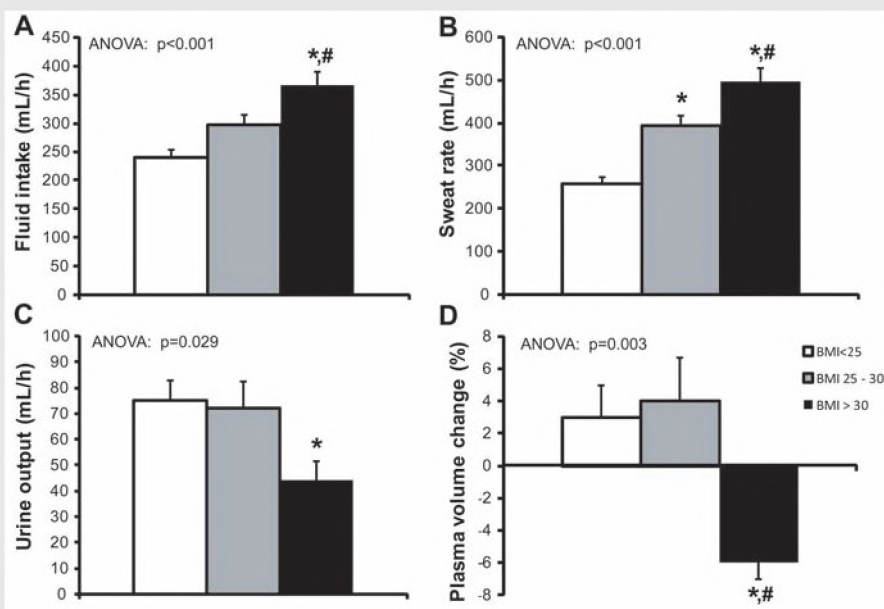
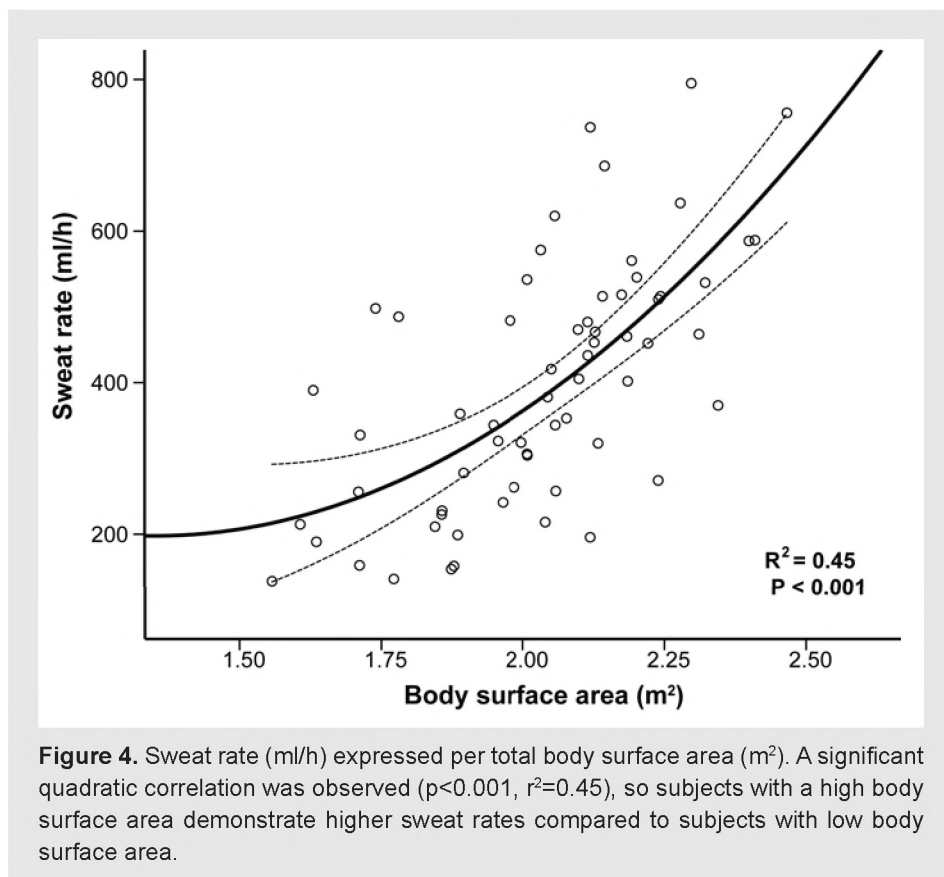


Figure 3. Fluid balance parameters (A: fluid intake, B: sweat rate, C: urinary output and D: plasma volume changes) presented per BMI group. Obese subjects showed a significantly higher fluid intake and higher sweat rate compared to overweight and lean subjects. Furthermore, obese subjects demonstrated also a significant lower urine output and decline in plasma volume compared lean peers. Data is presented as mean \pm SE. *,# Post hoc significant from lean (*) and overweight (#) subjects respectively.

obese (226 ± 70 mL/h/m²) subjects still demonstrated significantly higher sweat rates than lean subjects (133 ± 36 mL/h/m²) after correction for body surface area. Urine output in obese subjects was significantly lower than in lean subjects (Figure 3). Moreover, the incidence of post exercise high urinary specific gravity (≥ 1.030 g/mL), i.e. a marker for dehydration, was significantly higher in obese subjects ($OR_{\text{obese}} = 2.9$, $CI = 1.0-8.1$, $P = 0.044$) compared to the lean controls (Table 2), while no groups differences were found at baseline ($p = 0.53$). Whereas obese subjects demonstrated a significant decrease in plasma volume after exercise ($p = 0.001$), lean ($p = 0.20$) and overweight ($p = 0.21$) subjects demonstrated no change in plasma volume (Figure 3). Furthermore, overweight and obese subjects demonstrated a significantly larger decrease in body weight after exercise than the lean participants, but no differences were found when presented in relative terms (Table 2). Using body weight as a marker for dehydration, 20% of the total population met the criteria of dehydration, whilst no differences across groups were observed (Table 2).



Plasma sodium concentration

Lean subjects demonstrated no change in their plasma sodium concentration after prolonged moderate-intensity exercise (Figure 5). In marked contrast, a significant increase in post-exercise plasma sodium levels was observed in overweight and obese subjects (Figure 5). Whilst 13% of our subjects demonstrated post-exercise hypernatremia, the incidence was not different among lean ($n=2$, 7%), overweight ($n=4$, 14%) and obese subjects ($n=5$, 16%).

Discussion

This is the first study to examine the impact of obesity on physiological responses during prolonged moderate-intensity exercise. Walking duration, relative exercise intensity and training status were comparable across groups. We found that obese subjects tended to have a higher maximum core body temperature than

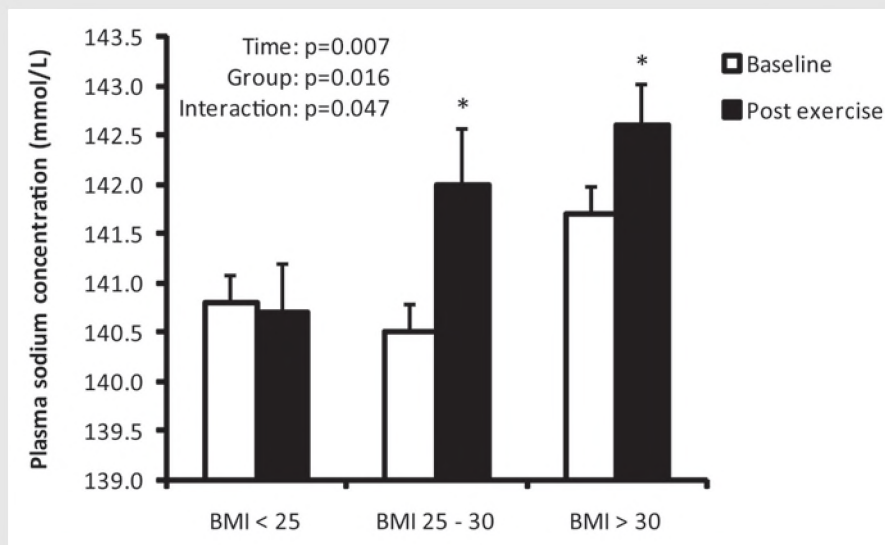


Figure 5. Plasma sodium concentration in lean (BMI<25), overweight (BMI 25-30) and obese (BMI>30) subjects at baseline and directly post-exercise. Two-way repeated measures ANOVA revealed a significant time, group and interaction effect. Data is presented as mean \pm SE. *Post hoc significant from baseline and significantly larger than the change in BMI<25.

lean controls. Furthermore, obese subjects demonstrated higher sweat rates, lower urine outputs and a higher fluid intake compared to their lean peers, accompanied by a larger decrease in body mass, decline in plasma volume, higher urine specific gravity and higher plasma sodium levels. These group differences suggest that overweight, but especially obese subjects, are prone to develop fluid and sodium imbalances.

Participants walked at an average exercise intensity of 72% of their maximal predicted heart rate, which is comparable with $\sim 60\%$ of their $\text{VO}_{2\text{max}}$.^{37, 38} Although the exercise intensity was not different across BMI groups, we found significant differences in the time course of core body temperature. All groups demonstrated a relatively large increase during the first 5 km, followed by a slow, but gradual increase thereafter. Interestingly, the lean participants demonstrated a lower rate of increase in core body temperature than overweight and obese participants. This is in agreement with previous observations of a larger change in core body temperature in obese subjects compared to lean subjects when exposed to a comparable increase in physical activity level.²³ Since sweating is the body's principal cooling mechanism,^{18, 21} obese subjects may also have an

increased fluid loss. Indeed, obese subjects demonstrated a significantly higher sweat rate compared to lean subjects. While the higher sweat rate in obese subjects was partly related to their larger body surface area (Figure 4),²² the sweat rate expressed per square meter body surface area (ml/h/m^2) was still significantly higher in overweight and obese subjects.

The higher sweat rate in obese subjects was accompanied by a lower urine output and higher fluid intake compared to lean subjects. An explanation for the lower urine output in obese subjects may relate to arginine vasopressin secretion. Arginine vasopressin is a hormone that stimulates water reabsorption in the kidneys, and may therefore contribute to a lower urine output. Although we have not measured arginine vasopressin levels, previous studies demonstrated that an increased serum osmolality or a decreased plasma volume stimulate the secretion of arginine vasopressin.^{39, 40} Therefore, the decreased urinary output in obese subjects in our study might be the consequence of their higher sodium levels and decreased plasma volume (i.e. more dehydrated status) compared to lean counterparts.

The higher fluid intake in obese subjects may also relate to the thirst stimulus. Previous studies showed that an increased serum osmolality or a decreased plasma volume stimulates the subject's thirst perception.^{41, 42} The significantly higher sodium levels in obese subjects, and thus higher osmolality, may have induced an increase in the thirst stimulus. In parallel, the decline in plasma volume, potentially followed by the release of angiotensin, could stimulate thirst perception as well. Furthermore, obese subjects had a slightly larger intake of sports drink compared to their overweight counterparts (18% versus 8% respectively). Taken together, the increased fluid intake and larger part of sports drinks in combination with a reduced urine output may enable obese subjects to compensate for the higher sweat rates in an attempt to regulate fluid balance.

In contrast to the larger variation in fluid intake and output in obese subjects, the incidence of dehydration, as defined by body mass change, was not different between lean, overweight and obese subjects. The identification of dehydration as applied in our study (body mass loss of $\geq 2\%$) is based on athletes.^{20, 28} In athletes, 2% body mass loss equals a 3% total body water loss.¹⁷ However, as fluid loss will predominantly occur from the lean body mass compartment, body composition may importantly impact the identification of dehydration. Adipose tissue consists of $\sim 10\%$ water, whereas fat-free tissue (e.g. muscle tissue) consists of 70-80% water.⁴³ Therefore, a 2% body mass loss in an obese individual (e.g. 110 kg, 46% body fat percentage), equals 4.5% loss of total body water content.⁴⁴ Thus, the classification of dehydration as $>2\%$ body mass loss may in fact underestimate the true presence of dehydration in obese and overweight subjects. To support this notion we have also examined post-exercise urinary specific gravity, which represents a useful method to identify dehydration.^{21, 29, 45} Interestingly, using

this measure, obese subjects had a ~3 times higher risk to develop dehydration compared to lean subjects. This emphasizes that one should be careful with interpreting body mass changes in overweight and obese subjects.

Whereas lean subjects were able to maintain their plasma-sodium concentration during exercise, a significant increase was observed in overweight and obese subjects. These marked differences between groups may be explained by hypertonic sodium gain or net water loss.³¹ Hypertonic sodium gain usually results from clinical interventions, but sodium loading (e.g. ingestion of sodium chloride) is also accidentally reported.³¹ Nevertheless, it is unlikely that overweight and obese subjects have consumed substantial amounts of sodium that can explain our findings. Alternatively, the increase in plasma sodium concentration may relate to the higher sweat rates in overweight and obese subjects (see above) compared to lean subjects. Plasma volume loss through sweating with a preserved amount of sodium, consequently leads to an increase in sodium levels. Taken together, our results show that obese subjects respond differently to prolonged moderate-intensity exercise under moderate ambient conditions compared to lean peers.

Clinical relevance. Moderate-intensity exercise is routinely prescribed as a effective strategy to lose weight and improve cardiovascular health.¹⁴⁻¹⁶ Although exercise prescription to obese and overweight subjects includes shorter bouts of exercise compared to this study, our data are clinically relevant given the popularity of prolonged walking (e.g. hill walking, pilgrimages, organized marches). Especially since we found in a recent study (unpublished data) that 40% of all participants of the Nijmegen Four Days Marches were overweight or obese. Nonetheless, all subjects were able to complete a prolonged exercise bout at ~72% of their maximal heart rate, whilst no clinical signs or problems were observed. This indicates that moderate-intensity exercise, even in prolonged settings, is safe and well tolerated by overweight and obese subjects. However, obese subjects demonstrated different physiological responses compared to their lean peers. This suggests that obese subjects may be at risk to develop health problems related to thermoregulation, fluid- or sodium balance under more strenuous environmental conditions. Therefore future fluid replacement guidelines should take BMI into account as a potential modifying factor.

Limitations. The strengths of this study are the inclusion of a large group of participants, the unique study design and completion of a prolonged exercise bout. However, some limitations should also be taken into account. First, BMI was used to define the lean, overweight and obese subjects. Although BMI provides no information regarding body composition, additional measurements revealed that body fat percentage, body surface area and waist-to-hip ratio differed significantly across groups. Therefore, we successfully included three different subgroups of obesity. Secondly, we estimated fluid loss by the assessment of urinary output and sweat excretion, thereby ignoring respiratory and gastrointestinal

fluid losses.^{46, 47} However, metabolic water production in the muscles has been shown to compensate for respiratory fluid loss,^{47, 48} whilst gastrointestinal losses are normally negligible (i.e. 100 mL/day).⁴⁶ Therefore, urinary excretion and sweat loss are considered the major determinants of total body fluid loss.^{17, 45, 47} Finally, urine specific gravity was used as marker of the fluid balance. Although this parameter can be influenced by large molecules (e.g. with albuminuria) and may be subordinate to other fluid balance parameters (plasma osmolality / urinary sodium concentration), this measure is easy to apply in field settings and provides valid information about the fluid status of a participant.⁴⁹ Despite potential limitations, the larger prevalence of high urine specific gravity levels in obese subjects was in agreement with the general finding of fluid imbalance in subjects with obesity.

In conclusion, obese subjects demonstrated higher sweat rates, lower urine outputs and a higher fluid intake compared to their lean peers during prolonged moderate-intensity exercise under moderate ambient conditions. The differences in fluid balance were accompanied by a larger decrease in body mass, higher urine specific gravity levels, higher plasma sodium levels and a decline in plasma volume in obese versus lean subjects. These changes suggest that overweight, but especially obese subjects, have an increased risk to develop fluid and sodium imbalances. To prevent impaired aerobic exercise performance levels and potential health problems, obese subjects should be advised to take precautions; in particular during exercise in strenuous environmental conditions.

Acknowledgement

We recognize the excellent help of the organization of the International Nijmegen Four Days Marches, Siemens Medical Solutions Diagnostics B.V. (Breda, The Netherlands), Noortje van Duijnhoven, Ralph Scholten, Madelijn Sander, Bregina Kersten, and Linda Pardoel. Dr. Thijssen is financially supported by the Dutch Heart Foundation (E. Dekker-stipend).

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Total body fluid (42 L)

Intracellular

Interstitium

Extracellular

Interstitium

Plasma

Cell body temperature

Distance
Group
Interstitium

NaCl

Chapter 5

Effect of Prolonged Walking on Cardiac Troponin Levels

Thijs M.H. Eijssvogels
Keith George
Rob Shave
David Gaze
Benjamin D. Levine
Maria T.E. Hopman
Dick H.J. Thijssen

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Abstract

Increased cardiac troponin I (cTnI), a marker for cardiac damage, has been reported after strenuous exercise in young subjects. However, little is known about changes in cTnI after moderate-intensity exercise in a heterogeneous population or which factors may contribute to this change in cTnI-levels. We examined cTnI-levels before and immediately after each day of a 4-day long-distance walking event (30-50 km/day) in a heterogeneous group (67 men, 42 women), across a broad age range (21-82 years), with known cardiovascular pathology or risk factors present in many individuals (n=24). Walking was performed at a self-selected pace. cTnI was assessed using a standard system (Immulin) with high values (≥ 0.20 $\mu\text{g/L}$) cross-checked using a high-sensitive cTnI-assay (Centaur). Mean cTnI-levels increased significantly from 0.04 to 0.07 $\mu\text{g/L}$ on Day 1, with no further increase thereafter (ANOVA; $P < 0.001$). Backward linear regression found a weak, but significant association of age ($p < 0.001$), walking speed ($p = 0.02$) and cardiovascular pathology ($p = 0.03$) with post-exercise cTnI-level (combined $r^2 = 0.11$, $P < 0.001$). In six participants (6%), cTnI was elevated above the clinical cut-off value for myocardial infarction on ≥ 1 day. These participants supported the regression analysis, as they were older, walked at higher relative exercise intensity, and reported a high prevalence of cardiovascular pathology. In conclusion, prolonged, moderate-intensity exercise may result in an increase in cTnI-levels in a broad spectrum of individuals, especially in older subjects with pre-existing cardiovascular disease or risk factors.

Introduction

The presence of intracellular cardiac troponin subunits T and I (cTnT and cTnI) in the blood is a sensitive and specific indicator for myocardial injury.^{1, 2} In recent years, elevated circulating cTnT/cTnI concentrations have been reported after prolonged exercise.²⁻⁶ The purpose of our study was to examine baseline and post-exercise cTnI-levels in a large, heterogeneous group of participants undertaking the Nijmegen Four Days Marches (the Netherlands), an annual 4-day walking event involving ~40,000 participants. Participants walked 30, 40 or 50 km on 4 consecutive days. Specifically, we examined changes in absolute cTnI-levels, identified those with an elevation in cTnI above the cut-off value for acute myocardial infarction (AMI), and examined whether post-exercise cTnI-levels were related to age, gender, body mass index (BMI), relative exercise intensity, core temperature, walking speed, training status or underlying cardiovascular pathology.

Methods

Subjects

A total of 109 participants (21 - 82 yrs) were randomly selected 4 weeks prior to the event (Table 1). All participants completed a minimum distance of 30, 40 or 50 km on 4 consecutive days. The Medical Ethical Committee of the Radboud University Nijmegen Medical Centre approved the study and all participants provided written informed consent prior to participation. This study was conducted in line with the Declaration of Helsinki.

Experimental design

One or two days prior to the start (Day 0), venous blood was drawn and serum was stored for later analysis, and general demographic data were obtained. Before the start of each walking day (which varied between 4 and 7 am depending on the walking distance) and at every 5 km point, heart rate and core body temperature were measured. Immediately after finishing, all measurements from Day 0 were repeated.

Measurements

All measurements were performed in the same laboratory located at the finish area. Measurements were performed between 11 am - 5 pm on Day 0, and directly after finishing (which varied between 12 - 5 pm) for subsequent walking days. The sequence of measurements was similar each day.

Subject characteristics. On Day 0, body weight (Seca 888 scale, Hamburg, Germany) and height were measured. In addition, resting heart rate and blood pressure were measured using an automated sphygmomanometer (M5-1

intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min seated rest.

Core body temperature. Core body temperature was assessed using a portable telemetry system (CorTemp™ system, HQ Inc, Palmetto, USA), which has been demonstrated to be safe and reliable.^{7, 8} Participants ingested an individually calibrated telemetric temperature sensor the evening preceding Day 1. Prior to the start of each walking day (Day 1-4), core temperature of each individual participant was measured using an external recorder. Baseline core body temperature was defined as the average of 3 consecutive measurements. Similarly, core body temperature was measured every 5 km along the route. Participants ingested a new telemetric sensor when the sensor was eliminated from the body or the transmitted signal was too weak to record. Mean core body temperature during each day was calculated as the average of all measurements, excluding the values derived before the start and after the finish. In addition, the highest value of these measurements was presented as the peak core body temperature.

Exercise intensity. Heart rate was measured with a 2-channel ECG chest band system (Polar Electro Oy, Kempele, Finland) simultaneously with core body temperature, using the same data recorder. Mean heart rate during each walking day was calculated as the average heart rate, excluding the values derived directly before the start and after the finish. Mean heart rate during exercise was presented in absolute values (beats per minute), but also as a percentage of the predicted maximal heart rate (% from $208 - 0.7 \cdot \text{age}$).⁹

Cardiac Troponin I. Ten ml of blood was drawn from an antecubital vein. Post-exercise on days 1-4, this was performed 10-20 min after the finish. Whole venous blood was collected in serum-gel vacutainer tubes and allowed to clot for ~45 min. After centrifugation, serum was aliquoted, frozen and stored at -80°C for later analysis. cTnI was analysed using the STAT Troponin I assay for the Immulite 2500 system (Siemens Healthcare Diagnostics, Breda, the Netherlands). Total assay imprecision was 7.8% at 2.3 µg/L and 7.9% at 29.1 µg/L. The detection level of this assay was set at 0.1 µg/L. However, quality control analysis of our data below 0.1 µg/L revealed a coefficient of variation (CV) of 8.1% and 14.7% at 0.08 and 0.02 µg/L, respectively, which then increased to 24.1% at 0.01 µg/L. Analysis was performed on a single day using the same calibration and set-up to minimize variation. After identifying participants with a cTnI above 0.2 µg/L, which is used as the clinical cut-off value for diagnosis of AMI, values were cross-checked using a highly-sensitive cTnI-assay (Centaur TnI-Ultra, Siemens Healthcare Diagnostics, Breda, the Netherlands). The assay imprecision of the highly-sensitive cTnI-assay was 5.3% at 0.08 µg/L and 3.0% at 27.2 µg/L, with a detection limit of 0.006 µg/L.

Table 1. Subject characteristics and details about the presence of (cardiovascular) pathology, presented for the entire group (n=109) and subdivided for participants that walked 30 (n=35), 40 (n=45) or 50 (n=29) per day.

	Overall	30 km	40 km	50 km
Demographic characteristics				
Male : female (n)	67 : 42	22 : 13	28 : 17	17 : 12
Smoking (n)	20 (18%)	3 (9%)	9 (20%)	8 (28%)
Age (years)	57 ± 15	69 ± 6	54 ± 16	48 ± 15
Length (cm)	174 ± 10	171 ± 9	175 ± 10	176 ± 10
Weight (kg)	77 ± 15	73 ± 12	78 ± 15	80 ± 17
Body Mass Index (kg/m ²)	25.2 ± 3.3	24.9 ± 2.6	25.0 ± 3.2	25.7 ± 4.1
Systolic blood pressure (mmHg)	138 ± 18	144 ± 19	135 ± 16	137 ± 19
Diastolic blood pressure (mmHg)	84 ± 10	86 ± 11	83 ± 9	84 ± 11
Previous participation (n)	94 (87%)	35 (100%)	35 (78%)	25 (86%)
Distance trained (km)	491 ± 661	541 ± 458	509 ± 866	398 ± 476
Exercise (h/week)	3.3 ± 4.3	3.9 ± 6.0	3.3 ± 3.3	2.6 ± 3.2
≥5 times/week ≥30 min exercise	91 (83%)	29 (83%)	37 (82%)	25 (86%)
Cardiovascular disease	24 (22%)	15 (43%)	8 (18%)	1 (3%)
Hypertension	22 (20%)	13 (37%)	8 (18%)	1 (3%)
Hypercholesterolemia	19 (17%)	6 (17%)	7 (16%)	6 (21%)
Myocardial / Cerebrovascular infarction	4 (4%)	4 (11%)	1 (2%)	0 (0%)
Diabetes Mellitus type II	2 (2%)	2 (6%)	0 (0%)	0 (0%)
Depression / asthma / rheumatoid arthritis	5 (5%)	2 (6%)	1 (2%)	2 (7%)
Medication use	35 (32%)	17 (49%)	13 (29%)	5 (17%)
Anti-hypertensiva	20 (18%)	14 (40%)	6 (13%)	0 (0%)
Statins	14 (13%)	6 (17%)	6 (13%)	2 (7%)
Diuretics	8 (7%)	6 (17%)	2 (4%)	0 (0%)
Anti-inflammatory drugs	(9%)	0 (0%)	(15%)	(10%)

Hypercholesterolemia is defined as those who have total cholesterol levels of >6.5 mmol, as previously diagnosed by a physician. Data is presented as mean ± standard deviation.

Plasma volume. Another 2 ml of blood was drawn from the antecubital vein for immediate analysis from the collecting syringe for plasma levels of hematocrit (Hct, in L/L) using a Rapidpoint® 400 (Siemens Healthcare Diagnostics Inc., Tarrytown, NY, U.S.A.). In addition, haemoglobin (Hb, in mmol/L) was determined

using a B-Hemoglobin analyzer (HemoCue AB, Ängelholm, Sweden). Relative changes in plasma volume (ΔPV , in %), were calculated from blood hematocrit and haemoglobin concentrations using Dill and Costill's equation.¹⁰

Ambient conditions. Dry bulb, wet bulb, and globe temperatures were measured every 30 minutes during the four days using a portable climate monitoring device (Davis instruments inc., Hayward, U.S.A.) positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula: $WBGT = 0.1 * (T_{\text{dry bulb}}) + 0.7 * (T_{\text{wet bulb}}) + 0.2 * (T_{\text{globe}})$.

Statistical analysis

Statistical analysis were performed using SPSS 16.0 (SPSS, Chicago, Illinois) software. All data are reported as mean (SD) unless stated otherwise, while statistical significance was assumed at $P < 0.05$. When data demonstrated a non-Gaussian distribution, Ln-transformation was applied. Repeated measures ANOVA (with Day as the independent factor) was used to assess differences across the 5 testing days for cTnI. Post-hoc *t*-tests with the Least Square Difference correction for multiple comparisons were performed when the ANOVA reported a significant main or interaction effect. Backward stepwise linear regression analysis was used to identify factors that significantly relate to post-exercise cTnI-levels. Age, gender, BMI, walking speed, core body temperature, distance trained, and cardiovascular pathology were examined as potential determinants of post-exercise cTnI-level.

Results

Due to orthopedic problems, 3 participants did not complete Day 1, while 2 participants did not finish on Day 2. Another participant was excluded from further participation as he exceeded the time limit on Day 2. As a result, 103 participants completed the event (94.5%), which was slightly greater than the overall completion rate (89.4%). Apart from age, baseline characteristics were not significantly different between the 3 groups that walked 30, 40 or 50 km (Table 1). Based on the medical history, 22% was diagnosed a priori with cardiovascular disease, with hypertension reported most frequently (Table 1). The 30-km group, including the oldest participants, reported the highest prevalence of cardiovascular disease (Table 1). Participants with prescribed medication predominantly used anti-hypertensive drugs, statins and/or diuretics (Table 1).

Exercise characteristics

Exercise was performed under mild ambient conditions, which did not differ across the 4 days (Table 1). Mean walking speed and mean finish time did not differ across days (Table 2). Core body temperature and heart rate increased significantly during exercise (Table 2). When presented as the relative intensity,

Table 2. Details about walking (average time and speed), ambient conditions (minimum and maximum temperature), and changes in physical parameters (absolute and relative mean heart rate, mean and peak core body temperature) for all participants (n=109) across the 4 walking days.

	Day 1 (n=106)	Day 2 (n=103)	Day 3 (n=103)	Day 4 (n=103)
Walking				
Time (hh:mm)	8:37 ± 1:38	8:57 ± 1:33	8:36 ± 1:47	9:08 ± 2:09
Speed (km/h)	4.6 ± 0.6	4.4 ± 0.6	4.6 ± 0.6	4.4 ± 0.6
Ambient conditions				
Minimum WBGT (°C)	13.2	14.2	12.3	12.5
Maximum WBGT (°C)	20.6	20.4	19.8	19.4
Physical parameters				
Heart rate (bpm)	115 ± 18	108 ± 16	105 ± 15	104 ± 14
Heart rate (% from HR _{max})	72 ± 10	68 ± 9	66 ± 10	65 ± 9
Maximum T _c (°C)	38.2 ± 0.4	38.3 ± 0.5	38.0 ± 0.5	38.1 ± 0.4
Plasma volume change (%)	0.2 ± 8.7	2.1 ± 8.1	9.2 ± 10.7	10.8 ± 10.8

Data is presented as mean ± standard deviation. WBGT, wet bulb globe temperature; bpm, beats per minute; HR_{max}, calculated maximum heart rate; T_c, core body temperature.

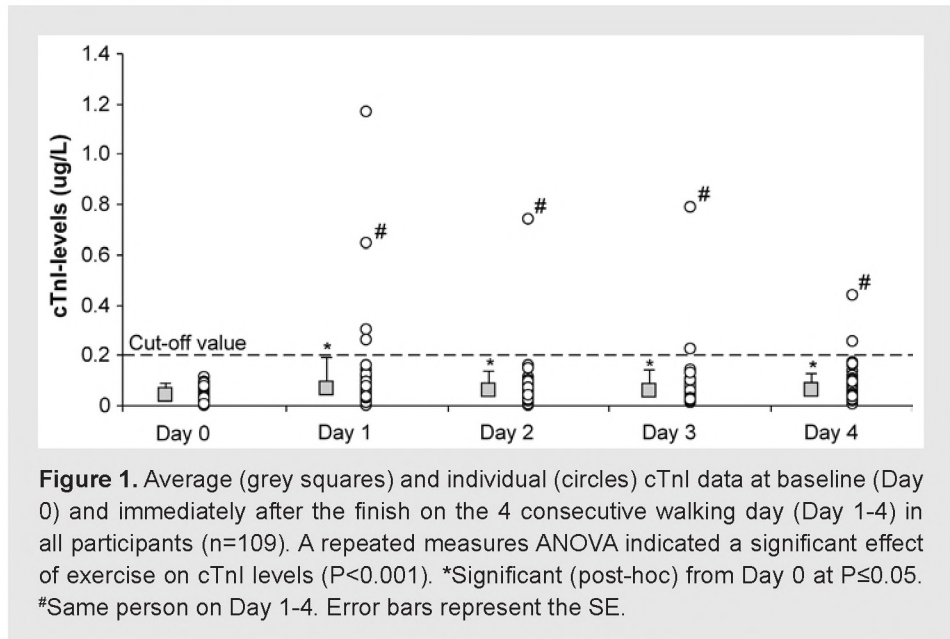
exercise intensity on the 4 days varied between 72% to 65% of their predicted maximal heart rate. Plasma volume did not change after Day 1 or 2, but showed a significant increase on consecutive days (Table 2, ANOVA: $P < 0.001$, post-hoc analysis: $P < 0.05$).

Cardiac troponin I

Natural logarithmic transformation was applied to the cTnI data set for analysis, as a non-Gaussian distribution was found. A significant increase in cTnI was found between Day 0 and Days 1-4, with no significant differences across the 4 days of walking (Figure 1). To gain insight into the factors that may contribute to cTnI-release, a backward linear regression analysis was performed. The regression analysis identified age ($\beta = 0.29$, $P < 0.001$), cardiovascular pathology ($\beta = 0.12$, $P = 0.031$) and walking speed ($\beta = 0.12$, $P = 0.022$) as significant predictors of increased post-exercise cTnI-levels (all parameters: $r^2 = 0.11$, $P < 0.001$), whereas gender ($\beta = 0.09$, $P = 0.073$) and distance trained ($\beta = -0.10$, $P = 0.052$) did not reach significance level.

Five participants showed a 'positive' cTnI (above the AMI cut-off) on 1 single day (Day 1: $n = 3$; Days 3 to 4: $n = 1$), which was confirmed using a highly-sensitive cTnI-assay (Table 3). One subject showed a 'positive' cTnI on all 4 walking days

(Figure 1). Analysis of these participants revealed that gender distribution, BMI, medication use, core body temperature, and walking speed were comparable with the entire population (Table 3). However, age, relative exercise intensity and presence of cardiovascular disease of this subgroup was higher than reported for the entire group.



Discussion

Our results indicate that ~9 h moderate-intensity walking exercise significantly elevates cTnI-levels in asymptomatic, non-athletic populations diverse in age and physical activity level, without a cumulative effect over 4 consecutive days. Moreover, when using the cTnI cut-off level for diagnosis of AMI,¹ 6% of our participants reported a positive test on 1 or more days, without reporting clinical symptoms or signs of AMI. Most importantly, we found that advanced age, walking speed and the presence of cardiovascular disease place individuals at greater risk for an increase in cTnI-levels after prolonged walking. Nonetheless, only a small portion of the cTnI elevation can be explained by these factors.

The increase in cTnI after each day of exercise is consistent with recent reports that exercise can elevate cTnI in asymptomatic humans.¹¹⁻¹⁴ However, our findings are novel in several ways. First, our participants performed moderate-intensity walking exercise in a temperate climate, while previous studies typically examined

Table 3. Details about participants (n=6) with a positive, clinically relevant, high sensitive cTnI test (99 percentile: 0.20 µg/L).

Subject (day)	cTnI (µg/L)	Age (years)	Sex (m : f)	BMI (kg/m ²)	Distance (km)	Fitness (sport hours/week)
1 (4)	0.25	57	F	25.9	50	5
2 (1)	0.30	53	F	30.9	50	4
3 (3)	0.20	82	M	24.7	30	0
4 (1)	1.17	79	M	26.2	30	4
5 (1)	0.26	74	M	21.5	30	1
6 (1)	0.65	75	F	26.9	30	2
(2)	0.74					
(3)	0.79					
(4)	0.44					
Average (n=6)		70	3:3	26.0		2.7
Average (n=109)		57	67:42	25.2		3.3

Subject (day)	cTnI (µg/L)	Speed (km/h)	Exercise intensity (%)	T _c (°C)	Pathology
1 (4)	0.25	4.0	73	-	Hypertension
2 (1)	0.30	5.4	87	38.6	None
3 (3)	0.20	3.7	71	37.9	None
4 (1)	1.17	3.1	75	37.9	Hypertension, COPD
5 (1)	0.26	4.7	83	37.9	Atherosclerosis
6 (1)	0.65	4.1	77	38.1	Hypertension
(2)	0.74	4.1	72	38.2	
(3)	0.79	4.6	71	38.2	
(4)	0.44	4.6	69	37.9	
Average (n=6)		4.3	75	38.1	78%
Average (n=109)		4.5	64	38.2	22%

BMI; body mass index, T_c; core body temperature, COPD; chronic obstructive pulmonary disease, cTnI; cardiac troponin I.

subjects after strenuous, high-intensity exercise such as marathon running or triathlon,¹¹⁻¹⁴ often performed under challenging conditions.^{15, 16} Despite these differences, our cTnI increase was comparable with a large cohort of runners (n=482) at the 2002 Boston marathon.¹⁷ Second, the exercise-induced elevation in cTnI-levels was comparable across the 4 subsequent walking days, without a time-dependent change in the number of positive tests. These findings indicate

no cumulative effect in cTnI-release, or short-term adaptations in cTnI-release, when exposed to the same exercise volume on 4 consecutive days. A recent paper also reported no consistent change in cTnI-levels on 22 days of repetitive cycling exercise in 10 healthy men.¹⁸ The moderate magnitude and short-term duration of post-exercise cTnI-elevation,¹⁹ but also rapid release and clearance, may contribute to this finding.

We also examined factors that might have contributed to cTnI-release and found walking speed to relate to post-exercise cTnI-levels, which reinforces recent observations after short-²⁰ or long-duration exercise.²¹ In addition, we identified, for the first time, advanced age and cardiovascular pathology to relate, at least partly, to higher post-exercise cTnI-levels. Even after excluding the 6 subjects with a 'positive' AMI cTnI-test in the analysis, similar results were observed. Although advanced age, walking speed and presence of cardiovascular pathology were related to post-exercise cTnI levels, only 11% of the elevation in cTnI can be explained. This indicates that exercise-induced cTnI-release is likely dependent on various, potentially interacting factors and as such is extremely difficult to predict.

Analysis of cTnI is recommended as a sensitive and specific marker for cardiac damage in the diagnosis of AMI.^{1, 2, 22} Although cTnI-levels were elevated after exercise, the average increase was modest ($\sim 0.03 \mu\text{g/L}$) and did not exceed the cut-off value for AMI in most subjects. The changes in cTnI may be related to exercise-induced reductions in plasma volume. However, no change in plasma volume was found on Day 1-2, and an approximate 10% increase in plasma volume was present on Day 3-4, making this possibility unlikely. A remarkable finding in our study is that 6% of our participants demonstrated cTnI-elevation above the AMI cut-off value on 1 or more days. Although clinically these cTnI-values may initially be of concern, subjects reported no clinical signs of (acute) cardiac damage.

Post-hoc analysis of the cTnI-positive group ($n=6$) revealed that presence of cardiovascular pathology and age were higher compared with the entire group ($n=109$, Table 3). This reinforces our previous findings from the multivariate analysis. Nonetheless, exercise-induced elevation in cTnI cannot be explained exclusively by these factors. Note that 5 subjects showed a 'positive' cTnI-test on a single day, while exercise and personal factors were not different from the other 3 days of the event. This emphasizes that prolonged exercise may result in a circulating cTnI value above the current clinical cut-off for AMI, which is modestly related, but not fully dependent, on a number of the factors that we examined.

Our findings raise an important question regarding the underlying mechanism for cTnI release. Possibly, cTnI release is related to the increase in myocardial work (and thus myocardial oxygen uptake) that is influenced by the exercise-related

increase in heart rate. Another potential mechanism relates to oxidative stress, as a recent study demonstrated that oxidative stress is strongly associated with an elevation in cTnI.²³ Since aging²⁴ and cardiovascular pathology²⁵ are related to increased oxidative stress, one may hypothesize that oxidative stress contributed to the increase in cTnI. Unfortunately, this remains unanswered in our study. Finally, myocardial damage could be triggered by various factors, including wall stress, coronary artery spasms, elevated stress on atherosclerotic plaques, and ischemia.²⁶ Nonetheless, the cTnI increase was small and not associated with symptoms of cardiac injury. Indeed, it may well be possible that the increase in cTnI did not reflect myocardial “damage”, but rather reflects an increase in membrane permeability during prolonged elevations in heart rate that, when accompanied by reduced renal blood flow during exercise, leads to a small increase in cTnI release and a decreased clearance. Our study cannot distinguish among these possibilities. Ultimately, it should be emphasized that exercise has important cardioprotective effects and this study should not be taken as evidence against the cardiovascular health benefits of (low intensity) physical activity.

A recent study reported a biphasic cTnT release during and after completion of a marathon in young men.¹² As little is known about the time course of post-exercise cTnI-release, it is possible that we measured cTnI during the nadir in some subjects. Moreover, various groups may differ in their exercise-induced cTnI-release kinetics or clearance. Accordingly, we may have underestimated the true prevalence of post-exercise elevations in cTnI above the clinical cut-off value.

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Total body fluid (42 L)

Intracellular

Interstitium 10.0 L

Extracellular

Interstitium 10.0 L

Plasma 3.0 L

Cell body temperature

Distance
Group
Interstitium



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Chapter 6

The impact of prolonged exercise and obesity on cardiac troponin levels in humans

Thijs M.H. Eijssvogels
Matthijs T.W. Veltmeijer
Keith George
Maria T.E. Hopman
Dick H.J. Thijssen

In revision



Abstract

Background: Elevated cardiac troponin I (cTnI), a marker for cardiac damage, has been reported after high-intensity exercise in healthy subjects. Currently, little is known about the impact of prolonged moderate-intensity exercise on cTnI release, but also the impact of obesity on this response.

Methods: 97 volunteers (55 men and 42 women), stratified for BMI, performed a single bout of walking exercise (30-50 km). We examined cTnI-levels before and immediately after the exercise bout in lean ($\text{BMI} < 25 \text{ kg/m}^2$, $n=30$, 57 ± 19 years), overweight ($25 \leq \text{BMI} < 30 \text{ kg/m}^2$, $n=29$, 56 ± 11 years), and obese subjects ($\text{BMI} \geq 30 \text{ kg/m}^2$, $n=28$, 53 ± 9 years). Walking was performed at a self-selected pace. cTnI was assessed using a high-sensitive cTnI-assay (Centaur; clinical cut-off value $\geq 0.04 \text{ } \mu\text{g/L}$). We recorded subject characteristics (body weight, blood pressure, presence of cardiovascular risk) and examined exercise intensity by recording heart rate.

Results: Mean cTnI-levels increased significantly from 0.010 ± 0.006 to $0.024 \pm 0.046 \text{ } \mu\text{g/L}$ ($P < 0.001$). The exercise-induced increase in cTnI was not different between lean, overweight and obese subjects (2-way ANOVA interaction; $P=0.27$). In 11 participants, cTnI was elevated above the clinical cut-off value for myocardial infarction. Logistic regression analysis identified exercise intensity ($P < 0.001$), but not BMI, body fat percentage or waist circumference to significantly relate to positive troponin tests.

Conclusion: Prolonged, moderate-intensity exercise results in a comparable increase in cTnI-levels in lean, overweight and obese subjects. Therefore, measures of obesity unlikely relate to the magnitude of the post-exercise elevation in cTnI.

Introduction

The presence of intracellular cardiac troponin subunits T and I (cTnT and cTnI) in the blood is a sensitive and specific indicator for myocardial injury.^{1, 2} Current guidelines of the European Society of Cardiology and the American College of Cardiology attribute a central role for elevated cTn levels in the diagnosis of acute myocardial infarction (AMI).^{1, 3} Previous studies have also reported release of circulating cTnT/cTnI concentrations after prolonged exercise, including moderate-intensity exercise,⁴ with cTnT/cTnI levels even exceeding the cut-off level typically used for the diagnosis of myocardial infarction.^{2, 5-8} The observation that elevated cTnT/cTnI levels relate to a physiological response to exercise or significant cardiac damage, makes interpretation of this test highly challenging and clinically relevant.

Previous studies indicated that cardiovascular risk factors, such as obesity, are associated with higher baseline troponin levels.⁹ As an inactive lifestyle contributes to the development of obesity and its related health problems,¹⁰⁻¹² exercise training is routinely prescribed to individuals with obesity. Since obese subjects demonstrated higher troponin levels at rest and are prone to the development of cardiovascular diseases,^{13, 14} obesity may alter the exercise-induced troponin release. Interestingly, a meta-analysis identified a positive relationship between body mass and post-exercise cTnT release.¹⁵ However, this relationship may be confounded by training status, body composition and exercise intensity. To date, no previous study directly assessed the impact of obesity on post-exercise cTnT/cTnI levels.

Therefore, the purpose of this study was to assess the effect of obesity on cardiac troponin release after prolonged moderate-intensity exercise. We measured cTnI concentrations in lean, overweight and obese subjects with comparable training status before and immediately after a single bout of prolonged exercise. For this purpose, we examined changes in absolute cTnI-levels, identified those with an elevation in cTnI above the cut-off value for acute myocardial infarction, and examined whether these post-exercise cTnI-levels were related to obesity or to other individual or exercise-related factors. We hypothesize to find higher cTnI levels in obese compared to overweight and lean subjects.

Methods

Subjects

Ninety-seven participants were included and stratified for body mass index (BMI), as this measure is frequently used and widely adopted to define overweight/obesity. Subjects were defined as lean (BMI < 25, n=30), overweight (25 ≤ BMI < 30, n=29) or obese (BMI ≥ 30 kg/m², n=28) (Table 1). In addition, we also

Table 1. Subject characteristics and details about the health status, presented per subgroup.

	BMI < 25	25 ≤ BMI < 30	BMI ≥ 30	P Value
Demographic characteristics				
Sex (men:women)	15:15	15:14	25:13	-
Age (yr)	57 ± 19	56 ± 11	53 ± 9	0.53
Height (cm)	174 ± 11	174 ± 9	175 ± 9	0.84
Weight (kg)	69.5 ± 11.2	83.5 ± 8.1 ¹	101.4 ± 10.7 ^{1,2}	<0.001
Body-mass index (kg/m ²)	22.8 ± 1.9	27.4 ± 1.4 ¹	32.9 ± 1.9 ^{1,2}	<0.001
Waist circumference (cm)	93 ± 6	102 ± 6 ¹	111 ± 7 ^{1,2}	<0.001
Waist to hip ratio	0.91 ± 0.07	0.95 ± 0.06	0.99 ± 0.07 ^{1,2}	<0.001
Body fat (%)	29 ± 6	34 ± 6 ¹	38 ± 6 ^{1,2}	<0.001
Glucose (mmol/L)	5.4 ± 1.3	5.4 ± 0.5	5.7 ± 1.4	0.41
Cholesterol (mmol/L)	5.3 ± 1.1	5.4 ± 1.1	5.5 ± 1.1	0.85
Triglycerides (mmol/L)	1.1 ± 0.6	1.5 ± 0.6 ¹	1.5 ± 0.8 ¹	0.069
HDL (mmol/L)	1.5 ± 0.3	1.2 ± 0.2 ¹	1.2 ± 0.3 ¹	0.001
LDL (mmol/L)	3.3 ± 0.9	3.6 ± 1.0	3.6 ± 1.0	0.54
Health status				
Mean distance trained (km/year)*	578 ± 651	452 ± 715	633 ± 587	0.54
Mean arterial pressure (mm Hg)	98 ± 11	104 ± 11	107 ± 13 ¹	0.011
Use of prescribed medicines (%)	47	62	82 ¹	
Diuretics (%)	3	3	16	
Anti-hypertensive drugs (%)	13	24	37 ¹	
Statins (%)	10	10	16	
Beta-blockers (%)	7	7	13	
Anti-diabetic drugs (%)	3	0	11	
Pathology (%)	53	72	68	
Hypertension (%)	20	24	37	
Hypercholesterolemia (%)	13	10	21	
Skin disease (%)	7	24	16	
Osteoporosis (%)	10	10	5	
Asthma (%)	3	14	16	
Diabetes (%)	3	0	11	

HDL, high density lipoproteins; LDL, low density lipoproteins. ^{1,2} = Post-hoc significant difference in relation to lean and overweight subjects respectively. * Ln-transformation was applied as a non-Gaussian distribution was present

assessed body fat percentage and waist circumference as alternative markers of overweight/obesity. Written informed consent was obtained from all participants prior to the start of the study. This study was approved by the Medical Ethical Committee of the Radboud University Nijmegen Medical Centre, and was conducted in accordance with the Declaration of Helsinki.

Experimental design

Subjects participated in the International Nijmegen Four Days Marches, an annual walking event in The Netherlands, and walked 30 km (30%), 40 km (47%) or 50 km (23%) at a self selected pace. Twelve to 36 hours prior to the start of the march, baseline measurements (subject characteristics, health and training status) were conducted under controlled conditions. Fifteen minutes prior to the exercise bout, heart rate and core body temperature were measured. During exercise, heart rate and core body temperature were measured every subsequent 5 km. Immediately after finishing, all measurements were repeated.

Measurements

Subject characteristics. At baseline, body mass (Seca 888 scale, Hamburg, Germany) and height were measured in duplicate and BMI was calculated. A four-point skinfold thickness measurement (biceps, triceps, sub-scapular, supra-iliac) was obtained by a well-trained and highly experienced researcher to calculate the body fat percentage.¹⁶ Waist circumference was measured midway between the lower rib margin and iliac crest. Hip circumference was measured at the level of widest circumference over the greater trochanters. Waist-to-hip ratio was calculated as waist circumference divided by hip circumference. Resting heart rate and blood pressure were measured twice using an automated sphygmomanometer (M5-1 intellisense, Omron Healthcare, Hoofddorp, the Netherlands) after 5-min seated rest. Finally, all subjects completed a questionnaire about their physical activity (hours of sport participation per week), training status (walking-specific training history in the year preceding the walking march) and health status (presence of pathology and use of medication).

Core body temperature. Core body temperature was assessed using a portable telemetry system (CorTemp™ system, HQ Inc, Palmetto, USA), which has been demonstrated to be safe and reliable.^{17, 18} Participants ingested an individually calibrated telemetric temperature sensor the evening preceding the experiment. Prior to the start of the exercise, core body temperature of each individual participant was measured using an external recorder. Baseline core body temperature was defined as the average of 3 consecutive measurements within 30 seconds. Similarly, core body temperature was measured at every 5 km point during the march. The highest value of these measurements was presented as maximum core body temperature.

Exercise intensity. Heart rate was measured with a 2-channel ECG chest band

system (Polar Electro Oy, Kempele, Finland) simultaneously with core body temperature (i.e. every 5 km point, 3 consecutive measurements which were taken within 30 seconds), using the same data recorder. Mean heart rate during exercise was calculated as the average heart rate, excluding the values derived directly before the start and after the finish. Exercise intensity was calculated by dividing the mean heart rate during exercise by the maximal predicted heart rate ($208 - 0.7 \cdot \text{age}$).¹⁹ Subsequently, exercise intensity was presented as a percentage of maximal heart rate ($\%HR_{\text{max}}$).

Blood analysis. Ten ml of venous blood were drawn from an antecubital vein at baseline and directly after finishing. Whole venous blood was collected in serum-gel vacutainer tubes and allowed to clot for ~45 min. After centrifugation, serum was aliquoted, frozen and stored at -80°C for later analysis. Analysis was performed on a single day using the same calibration and set-up to minimize variation. cTnI was analysed using a highly-sensitive cTnI-assay (Centaur TnI-Ultra, Siemens Healthcare Diagnostics, Breda, the Netherlands). The assay imprecision of the highly-sensitive cTnI-assay was 5.3% at $0.08 \mu\text{g/L}$ and 3.0% at $27.2 \mu\text{g/L}$. A cTnI-value of $0.04 \mu\text{g/L}$ is the clinical cut-off value for myocardial infarction.²⁰

Ambient conditions. Throughout the experiment, dry bulb, wet bulb, and globe temperatures were measured every 30 minutes using a portable climate monitoring device (Davis instruments inc., Hayward, U.S.A.) positioned at the start/finish area. The wet bulb globe temperature index (WBGT) was calculated using the formula: $\text{WBGT} = 0.1 (T_{\text{dry bulb}}) + 0.7 (T_{\text{wet bulb}}) + 0.2 (T_{\text{globe}})$.²¹

Statistical analysis

All values were presented as mean \pm standard deviation, unless indicated otherwise. Statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, Illinois). The level of statistical significance was set at $p < 0.05$. The normality of the data distribution was examined by the Kolmogorov-Smirnov test. When data demonstrated a non-Gaussian distribution, Ln-transformation was applied. Comparisons between groups were assessed using a one-way ANOVA for continuously distributed data. A 2-way repeated measures ANOVA was applied to examine the change in cTnI after exercise in the 3 distinct groups (exercise \times group). Post-hoc *t*-tests with the Least Square Difference correction for multiple comparisons were performed when the ANOVA reported a significant main or interaction effect. A backward stepwise linear regression analysis was used to identify factors that significantly relate to post-exercise cTnI-levels. We have included BMI, age, sex, exercise intensity and pre-existing cardiovascular pathology as potential determinants of post-exercise cTnI-levels as previous studies have demonstrated evidence that these factors may relate to exercise-induced cTnI release.^{4, 15} In addition, we replaced BMI with 2 alternative measures of obesity (fat percentage and waist circumference) in the regression analysis to

further assess the effect of obesity on post-exercise cTnI levels. Finally, using the same sets of parameters, a binary logistic regression analysis was used to identify factors that contribute to the positive cTnI-samples. The odds ratio (OR) and 95% confidence interval (CI) were presented for those factors that were identified as significant factors to contribute to a positive cTnI-sample.

Results

The three BMI groups were not different in age and height, but as expected differed significantly in weight, BMI, body surface area, waist circumference and body fat percentage (Table 1). Participation in sports activities and the distance trained was not different across groups (Table 1), but the average training distance of 560 km suggested that our subjects were well prepared for the walking march. No differences in the prevalence of (cardiovascular) pathology were found (Table 1). Obese subjects, however, showed a higher mean arterial pressure and used more prescribed medication than their lean counterparts, particularly anti-hypertensive drugs (Table 1).

Exercise characteristics

All subjects successfully completed the exercise bout. The WBGT increased from 14.0 °C in the morning to a maximum of 25.0 °C in the afternoon. Walking speed did not significantly differ across groups (Table 2). Baseline and maximum core body temperature during exercise were not different between lean, overweight and obese subjects, whilst also exercise intensity (%HR_{max}) was comparable across groups (Table 2).

Table 2. Exercise characteristics presented per BMI group.

	BMI < 25	25 ≤ BMI < 30	BMI ≥ 30	P Value
Exercise characteristics				
Exercise duration (hh:mm)	8:32 ± 1:58	8:39 ± 1:25	8:48 ± 1:23	0.79
Speed (km/h)	4.8 ± 0.7	4.7 ± 0.8	4.7 ± 0.7	0.69
Baseline T _c (°C)	37.5 ± 0.4	37.6 ± 0.5	37.5 ± 0.3	0.55
Maximum T _c (°C)	38.3 ± 0.3	38.4 ± 0.3	38.5 ± 0.3	0.053
Exercise intensity (%HR _{max})	71 ± 9	71 ± 10	74 ± 8	0.30

P-value refers to a One-Way ANOVA. T_c, core body temperature.

Cardiac troponin

Ln-transformation was applied to the cTnI data set, as a non-Gaussian distribution was found. Baseline cTnI levels were not different across the three groups.

The 2-way ANOVA revealed a significant increase in cTnI from 0.010 ± 0.006 to 0.024 ± 0.046 $\mu\text{g/L}$ after exercise ($P < 0.001$) (Figure 1). However, the magnitude of the exercise-induced increase in cTnI in lean (pre: 0.009 ± 0.007 , post: 0.014 ± 0.010 $\mu\text{g/L}$) was not different from overweight (pre: 0.012 ± 0.008 , post: 0.040 ± 0.085 $\mu\text{g/L}$) or obese subjects (pre: 0.009 ± 0.005 , post: 0.021 ± 0.016 $\mu\text{g/L}$) (Figure 1). To gain further insight into factors that contribute to cTnI-release, a backward linear regression analysis identified age ($\beta = 0.27$, $P = 0.003$), sex ($\beta = -0.19$, $P = 0.031$) and exercise intensity ($\%HR_{\text{max}}$, $\beta = 0.47$, $P < 0.001$, Figure 2) as significant predictors of post-exercise cTnI-level ($r^2 = 0.38$, $P < 0.001$), while pre-existing cardiovascular pathology was excluded from our model. Using fat percentage as a marker for obesity, we identified the same predictor variables with a comparable total predicted variance ($r^2 = 0.36$, $P < 0.001$). Also using waist circumference we found the same total predicted variance ($r^2 = 0.38$, $P < 0.001$), and confirmed the predictive capacity of age ($\beta = 0.31$, $P < 0.001$) and exercise intensity ($\%HR_{\text{max}}$, $\beta = 0.43$, $P < 0.001$). However, waist circumference ($\beta = 0.22$, $P < 0.01$) replaced sex as a significant predictor.

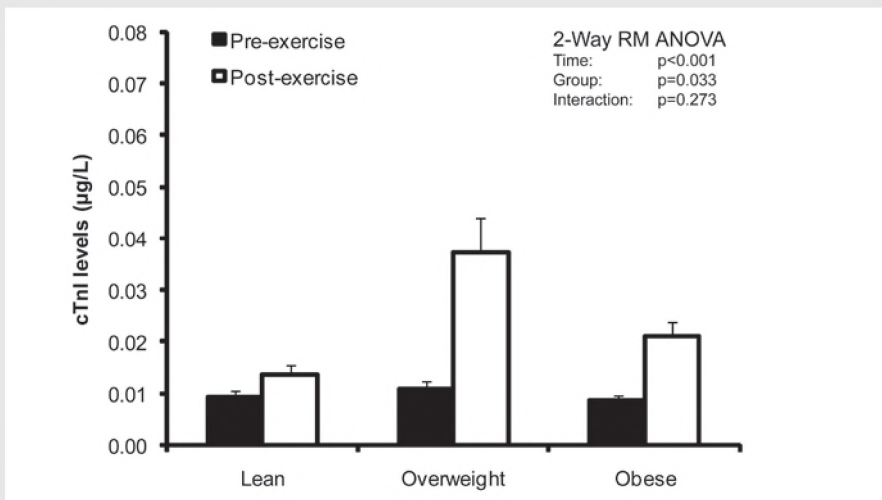


Figure 1. Pre- (black bars) and post-exercise (white bars) cTnI levels in lean (BMI < 25 kg/m^2 , $n = 30$), overweight ($25 \leq \text{BMI} < 30$ kg/m^2 , $n = 29$) and obese subjects (BMI ≥ 30 kg/m^2 , $n = 38$). Two-way RM ANOVA revealed a significant time ($p < 0.001$) and group ($p = 0.033$) effect. However, no interaction was present (time*group, $p = 0.27$) which indicates that the exercise-induced increase in cTnI was not significantly different across groups. Error bars represent SE.

Eleven of our participants (9 male, 2 female) showed a 'positive' cTnI level, e.g. above the AML cut-off. Although these subjects represented 25% of the overweight

and 11% of the obese groups, the odds ratio did not reveal a significant increased risk of BMI-group (lean, overweight or obese) on the presence of a 'positive' cTnI-test. In addition, subjects with a 'positive' and 'negative' cTnI level did not differ in BMI, fat percentage or waist circumference (corrected for sex). Using binary logistic regression analysis, we found that exercise intensity (%HR_{max}, $p < 0.01$, OR=1.1, CI: 1.0 – 1.3) was associated with a positive cTnI sample. This finding was present, independent of BMI, fat percentage or waist circumference as a measure of obesity in our model.

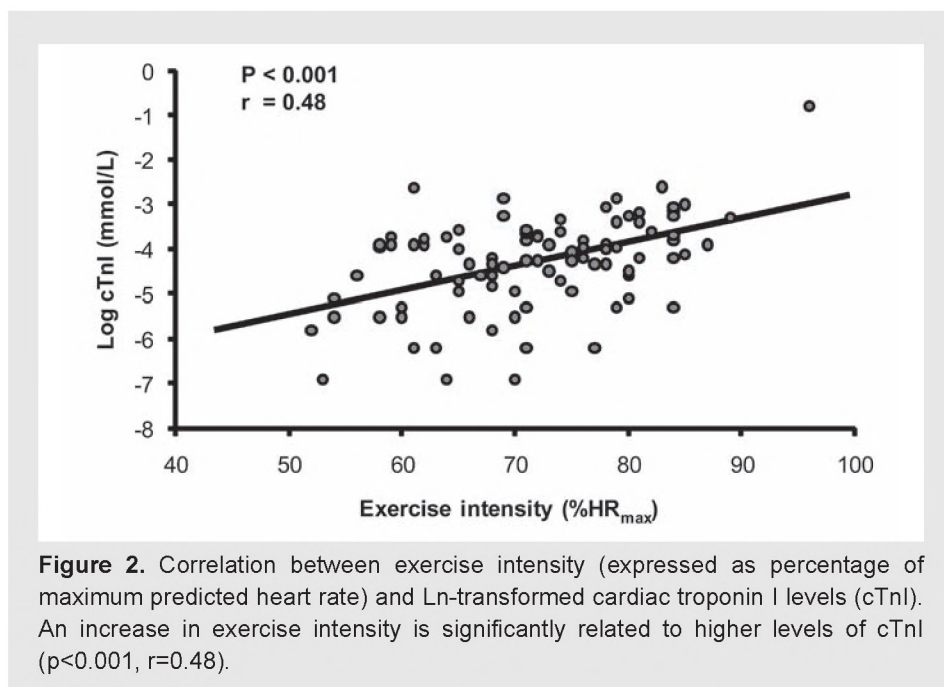


Figure 2. Correlation between exercise intensity (expressed as percentage of maximum predicted heart rate) and Ln-transformed cardiac troponin I levels (cTnI). An increase in exercise intensity is significantly related to higher levels of cTnI ($p < 0.001$, $r = 0.48$).

Discussion

This is the first study, to our knowledge, to directly examine the impact of obesity on cTnI-release during prolonged exercise. Our results indicate that prolonged moderate-intensity walking exercise significantly elevates cTnI-levels in asymptomatic subjects. More importantly, the magnitude of the absolute cTnI-increase was comparable between lean, overweight and obese subjects. When using the cTnI cut-off level for diagnosis of AMI,¹ 11% of our asymptomatic participants demonstrated a positive test. Nonetheless, our statistical analysis revealed that these positive cTnI tests cannot be explained through measures of obesity (BMI, body fat percentage or waist circumference).

Analysis of cTnI is recommended as a sensitive and specific marker for cardiac damage in the diagnosis of AMI.^{1, 2, 22} The observation that cTnI-levels were elevated after exercise in lean, overweight and obese subjects, with even 11% of our population (n=11) exceeding the cut-off value for AMI, is highly clinically relevant. Whilst previous studies predominantly focused on cTnI-levels in athletes after high-intensity exercise,²³⁻²⁶ we showed that prolonged moderate-intensity walking exercise (72% of the maximal predicted heart rate) can lead to significant elevations of post-exercise cTnI in a heterogeneous group of asymptomatic humans.

We found that the exercise-induced increase in cTnI is comparable between lean, overweight and obese subjects. This suggests that obesity does not impact the magnitude of post-exercise cTnI elevation. Indeed, linear regression analysis using various measures of obesity (i.e. BMI, fat percentage and waist circumference) identified that exercise intensity, age and sex significantly contribute to the post-exercise cTnI level. Although one of the models replaced sex with waist circumference, this finding may be confounded by the co-linearity between sex and waist circumference. Also, 9 of the 11 subjects with cTnI-levels above the clinical cut-off value were men who typically demonstrate a larger waist circumference than women. Furthermore, we demonstrated that BMI, body fat percentage and waist circumference were not different between subjects with and without a positive cTnI test, whilst the binary logistic regression analysis identified exercise intensity as the sole predictor of a positive cTnI sample. Taken together, our data suggests that obesity unlikely contributes to the magnitude of the post-exercise elevation in cTnI.

Although elevated cTnI levels are suggestive for cardiac damage, the average cTnI increase was small and was not associated with symptoms of cardiac injury. It may well be possible that the increase in cTnI did not reflect irreversible ischemic myocardial “damage”, but relates to a physiological response during prolonged exercise. The elevated heart rate during exercise may cause an increased mechanical stress on the heart, possibly leading to an increased release of cTnI. Interestingly, our study identified exercise intensity as the strongest predictor for the increase of cTnI levels, but also for a positive cTnI test. Exercise intensity, and the associated increased myocardial work, may therefore contribute to cTnI-release during prolonged moderate-intensity exercise. These findings are in agreement with a recent paper that found that exercise intensity influenced the magnitude of the troponin response during marathon running.²⁷ Another potential explanation relates to an impaired clearance of troponin from the circulation. Blood flow in the abdominal area and kidneys reduces markedly during exercise.²⁸⁻³⁰ Consequently, the kidneys capacity to secrete substances is attenuated,³⁰ possibly resulting in a small increase in cTnI concentration in the blood.

Limitations. The strengths of this study are the inclusion of a large group of participants, the unique study design and completion of a prolonged exercise bout. Due to practical limitations we did not determine maximal heart rate, but used a previously validated and frequently used model to predict maximal heart rate.¹⁹ A limitation is our single post-exercise assessment of cTnI, as previous observations reported a time-dependent change in cTnI after a marathon.²⁴ It is currently unknown whether obesity alters the time-course of cTnI-release after exercise and, therefore, potentially alters our findings. Also, it must be noted the coefficient of variation increases when measuring at the lower end of the cTnI spectrum. However, excluding subjects with low values (i.e. below 0.006 µg/L) did not alter the major outcomes of our study.

In conclusion, prolonged exercise resulted in a significant increase in cTnI-levels in asymptomatic lean, overweight and obese subjects. The magnitude of the exercise-induced increase in cTnI was not different among lean, obese and overweight subjects and relates predominantly to age, gender and exercise intensity. These findings were reinforced by our subjects with a cTnI above the clinical cut-off level, as their BMI, fat percentage and waist circumference was comparable with subjects below this level. Therefore, obesity unlikely contributes to the magnitude of the post-exercise elevation in cTnI.

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Total body fluid (42 L)

Intracellular

Interstitium

Extracellular

Interstitium

Plasma

Cell body temperature

Distance
Group
Interstitium

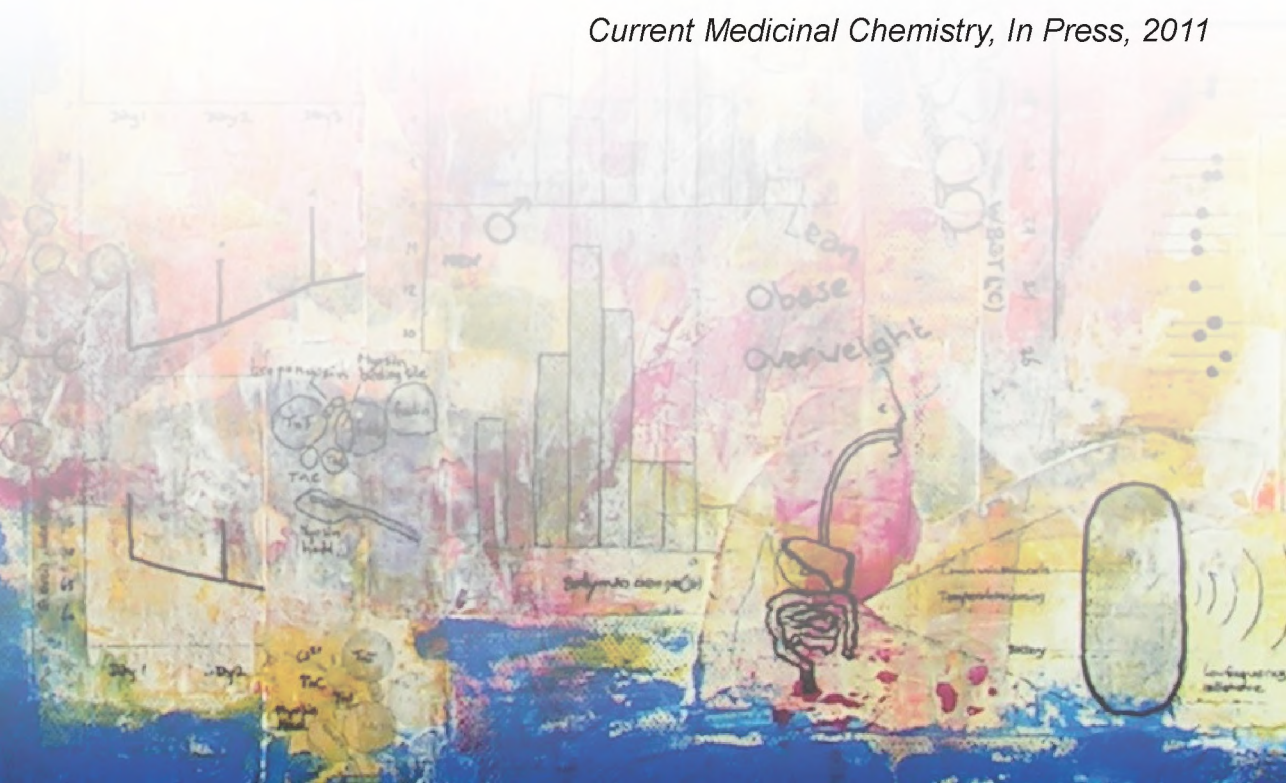
NaCl

Chapter 7

Exercise-induced cardiac troponin release: real-life clinical confusion

Thijs M.H. Eijssvogels
Rob Shave
Arie van Dijk
Maria T.E. Hopman
Dick H.J. Thijssen

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Abstract

Exercise training represents a successful and powerful strategy to prevent future cardiovascular disease. Paradoxically, performance of exercise is also associated with an increased risk of acute cardiac events. Accordingly, patients may present to hospital with cardiac symptoms following a bout of unaccustomed physical effort (e.g. exercise). Current guidelines for the identification of an acute myocardial infarction (AMI) importantly depend on the presence of cardiac troponin as a highly sensitive marker of cardiac damage. However, a number of studies have reported elevated cardiac troponin levels in asymptomatic, healthy subjects after endurance exercise (such as a marathon, prolonged cycling or prolonged walking). These observations indicate that elevated cardiac troponin levels can be the result of cardiac ischemia, and subsequent necrosis, but also may be related to strenuous exercise. In this paper, we present three different clinical cases of post-exercise elevations in cardiac troponins, each with a distinct clinical presentation. These case studies emphasize that a detailed assessment of all symptoms and a thorough patient-history are prerequisite for accurate interpretation of a positive cardiac troponin test following exercise.

Introduction

Exercise training represents a successful strategy to prevent future cardiovascular disease in healthy subjects and reduces the risk for progression and/or development of secondary problems in those with established cardiovascular pathology.¹⁻³ Paradoxically, performance of exercise is also associated with an increased risk of acute cardiac problems, especially in those with an increased cardiovascular risk and who perform sudden unaccustomed bouts of exercise.⁴ Following a suspected cardiac event, serum is analysed at admission for the presence of elevated cardiac troponin (cTn), either subunits T or I (cTnT and cTnI), as highly sensitive and specific markers of myocardial injury.⁵⁻⁷ According to current guidelines, an acute myocardial infarction is diagnosed if cTn levels are above the 99th percentile of the upper reference limit, combined with evidence of myocardial ischaemia with at least one of the following: (1) symptoms of ischaemia; (2) ECG changes indicative of new ischaemia; (3) development of pathologic Q waves in the ECG; (4) imaging evidence of new loss of viable myocardium or new regional wall motion abnormality.^{5, 8}

Interestingly, prolonged exercise in healthy, asymptomatic individuals has been shown to elevate cTn. Indeed, levels of cTn above the clinical cut-off used for the diagnosis of AMI have been shown following marathons, triathlons, cycling and/or walking events.⁹⁻¹³ Despite the post-exercise elevation in cTn, previous studies have not reported (patho)physiological evidence or clinical signs of myocardial injury. This suggests that post-exercise cTn levels may be related to a physiological rather than a pathological response after the exercise stimulus.¹⁰ Accordingly, the interpretation of elevations in cTn following unaccustomed exercise can be highly challenging and has important clinical implications. Differentiation between the physiological *versus* pathological release of cTn is important to avoid misdiagnosis. For example, a false-positive diagnosis could lead to unnecessary hospital admission and invasive investigations,¹⁴ while a false-negative diagnosis could be potentially life threatening for the patient. The recent development of even more sensitive cTn assays effectively reduces the incidence of false-negative tests in the *general* population. However, in individuals undertaking prolonged exercise, these new highly sensitive assays will better detect the small cTn release following exercise. In addition, relatively little is known about the challenging interpretation of cTn elevation in subjects after (strenuous) exercise, which may result in clinical confusion.

In this paper, we present three distinct case studies, each demonstrating elevation in cTn post-exercise, yet each presenting with different symptomatology. Discussion of these cases and recognition of the importance of other signs and symptoms may help clinicians and laboratories to improve the clinical management of “*positive*” cTn samples in people who have recently undertaken strenuous exercise.

Case presentation

The most important information regarding the exercise characteristics, clinical symptoms and laboratory tests is summarised in Table 1.

Case 1

A 59 year old male (1.86 m, 88 kg, BMI 25.4 km/m²) participated in the Nijmegen Four Days Marches, which included 40 km of walking exercise per day for four consecutive days. The event was held under moderate climate conditions (maximum ambient temperature of 25.0°C). The subject had no history of smoking, cardiovascular disease, cardiovascular risk factors and was not using any medication. The subject reported to be physically active on at least five days per week for at least 30-minutes. As part of a previously published scientific study,¹⁵ blood was drawn the day before the march and following completion everyday thereafter. cTnI-level was 0.025 µg/L before the march, which is under the clinical cut-off value for AMI (0.040 µg/L).¹⁶ The subject walked for ~7.5 hours (5.4 km.hour⁻¹) and with an average heart rate of 160 beats per minute on each of the four days. Core body temperature was measured using a wireless telemetry system and increased to an average finish temperature of 38.4°C, indicating that he was not hyperthermic.¹⁷ The subject did not report any physical complaints during or after the marches. Using a visual analogue scale (scale 0-10, with 10 as maximal effort), he rated the walking exercise as a 6. Post-exercise blood was taken 20-minutes after finishing each day. Analysis of stored serum samples, three-months after the event, revealed an increase in cTnI to 0.448 µg/L after day-1 and values of 0.314, 0.171 and 0.097 µg/L on the subsequent three days. As a final diagnosis, the elevation of cTnI-levels in this case were due to the preceding physical exercise.

Case 2

A 47 year old male (1.80 m, 72 kg, BMI 22.2 km/m²) was admitted to the Radboud University Nijmegen Medical Centre (16:00 h.) after completing a 15-km run. The race was completed under cool climatic conditions (maximal air temperature 9 °C), and he finished the race in 1 hour 12 minutes (12.5 km.hour⁻¹). No (family) history of myocardial infarction, stroke or other cardiovascular diseases was reported. The patient used no medications, oral supplements or tobacco while he routinely performed 4 hours of moderate- to high-intensity exercise per week.

The patient experienced nausea after finishing the 15-km the race and after ~60 minutes he collapsed and lost consciousness. After successful application of basic life support combined with 3 shocks from an automated external defibrillator (AED), vital functions were stable (pulse and breathing), but he was still unconscious. After 10 minutes, an ambulance arrived and he was transferred to hospital after intubation for mechanical ventilation and administration of aspirin and heparin intravenously. On admission (16:15 h.), ECG showed sinus rhythm

with maximum 2 mm ST-elevation in leads II-III-aVF and downsloping ST-segment depression in leads I, aVL, V2-V6, suggestive of an acute myocardial infarct. He was in sinus-tachycardia (110 bpm) and showed hypertension (160/114 mmHg). Cardiac auscultation was normal. Neurological examination revealed a Glasgow Coma Score of 1-2-tube. Laboratory tests immediately on admission showed cTnI below the detection limit ($<0.2 \mu\text{g/L}$). However, blood tests at 6.50 PM revealed cTnI-levels above this limit ($0.82 \mu\text{g/L}$), which demonstrated a further increase on subsequent days (5.82 , 11.30 , 17.40 and $16.60 \mu\text{g/L}$, respectively). Creatine kinase was increased immediately after admission ($172 \mu\text{g/L}$), and increased to 400 , 1392 , 1908 , 3010 and $3542 \mu\text{g/L}$ during subsequent laboratory tests at 18:50, 00:39, 0:52, 11:52 and 17.43 h., respectively. Shortly after admission (17:21 h.), he was taken for coronary angiography after administration of intravenous aspirin and 10,000 International Units (IU) of Heparin. Coronary angiography revealed an occlusion of the right coronary artery at the level of the right ventricular branch. After opening of the occlusion by primary stenting with a drug-eluting stent, a 70-80% stenosis at the origin of the right posterior descending artery partially consisting of dislodged thrombus became apparent. Balloon dilatation of this lesion in the posterior descending artery was subsequently performed. The left main coronary artery was normal, but significant proximal stenoses of the left anterior descending artery, the left circumflex artery and the left marginal artery were found. The patient was transported to the intensive care unit for mechanical ventilation, whole body cooling for 24 hours and pharmacological treatment, consisting of beta-blockade, ACE-inhibition, dual anti-platelet therapy (80 mg aspirin and 75 mg clopidogrel after a loading dose of 600 mg), heparin and a statin. Twelve days after admission, he was discharged from hospital. The final diagnosis for this case was an out-of-hospital cardiac arrest due to ventricular fibrillation in the setting of an acute myocardial infarction.

Case 3

A 31 year old male (1.86 m, 70 kg, 20.2 kg/m^2) was admitted to the Maasziekenhuis-hospital (18:30 h.) with a tight band feeling. He successfully completed four days of walking (50 km per day) during the Nijmegen Four Days Marches and reported no (family) history of myocardial infarction, stroke or other cardiovascular diseases. The patient used no medications, oral supplements, tobacco or caffeine. Furthermore, he was recreationally active (4 hours walking and 1 hour football per week) and frequently used a bicycle for transportation (3 hours per week). The walking march was held under mild to moderate climatic conditions (maximal temperatures $17\text{-}20^\circ\text{C}$), and he walked with an average speed of $4.7 \text{ km}\cdot\text{hour}^{-1}$.

The patient reported a tight band-feeling around his chest that started 1 hour prior to admission. The night before (03:30 h.), he experienced similar complaints, which disappeared after 45 min. On admission to a small community hospital, he was in sinus-bradycardia (58 bpm), exhibited normal breathing frequency and was normotensive ($127/78 \text{ mmHg}$). He reported no shortness of breath, nausea,

sweating, anxiety or chest pain. Auscultation and neurological examination revealed no abnormalities. ECG at admission did not show any signs of ischemia, pericarditis or right heart pressure overload (e.g. ST elevation or depression, T wave inversion, Q waves or PR depression). The tight band-feeling around his chest disappeared 30 min after admission. Laboratory tests showed elevated levels for creatine kinase-MB (32.0 µg/L) and cardiac troponin I (4.26 µg/L), while CRP levels were only mildly increased (12 mg/L) and the leukocyte concentration was normal ($5.6 \times 10^9/L$). He was taken to the intensive care unit for (overnight) monitoring, while standard oral pharmacological treatment was started (80 mg acetylsalicylic acid, 75 mg clopidogrel, 2x0.8 mg enoxaparine, 2x40 mg atorvastatin, 4x25 mg metoprolol). The day after admission, new laboratory tests (03:00, 09:15 and 16:45 h.), revealed a decrease in creatine kinase-MB levels (29.3, 18.8 and 13.0 µg/L, respectively), but no change in cTnI (4.21 µg/L at 16:45 h.). Subsequently, pharmacological treatment was stopped. Two days after admission, a 12-lead ECG showed no abnormalities at rest or during a graded-maximal cycling test. In addition, echocardiography showed no abnormalities. Three days after admission, the patient was discharged from the hospital and pharmacological treatment was stopped. After 9 months, additional tests were performed to examine potential scarring of the cardiac tissue. His resting ECG and lipid levels showed no abnormalities (total cholesterol 3.8 mmol/L, triglycerides 0.74 mmol/L, low-density lipoproteins 2.65 mmol/L, high-density lipoproteins 0.82 mmol/L). Furthermore, a cardiac MRI-scan revealed normal left ventricular mass and volume, and no regional wall motion abnormalities were present. In addition, no abnormalities were found during first-pass early perfusion MRI, and there was no delayed enhancement of the ventricular walls using gadolinium contrast. A final diagnosis for this patient remains debatable as we found no signs for cardiac damage or ischaemia, whilst elevated troponin levels are unlikely explained by the prolonged, multiple day, exercise stimulus.

Discussion

This paper presented 3 distinct clinical cases, each with an elevation in cTn following successful completion of a bout of prolonged moderate- to high intensity exercise. In this last section we will discuss the importance and recognition of the clinical symptoms in relation to changes in cTn. This information may help clinicians with decision making in relation to post-exercise elevations in cardiac troponins.

The first case reveals an increase in cTnI above the clinical cut-off value on 4 consecutive days when performing prolonged, moderate-intensity walking exercise. Nonetheless, this subject reported no physical complaints, clinical signs or specific symptoms of ischemia (Table 1). Current guidelines indicate that clinical

Table 1. Characteristics of three cases with elevated cardiac troponin levels after exercise with an individual score for the presence (+) or lack (-) for a certain marker for AMI.

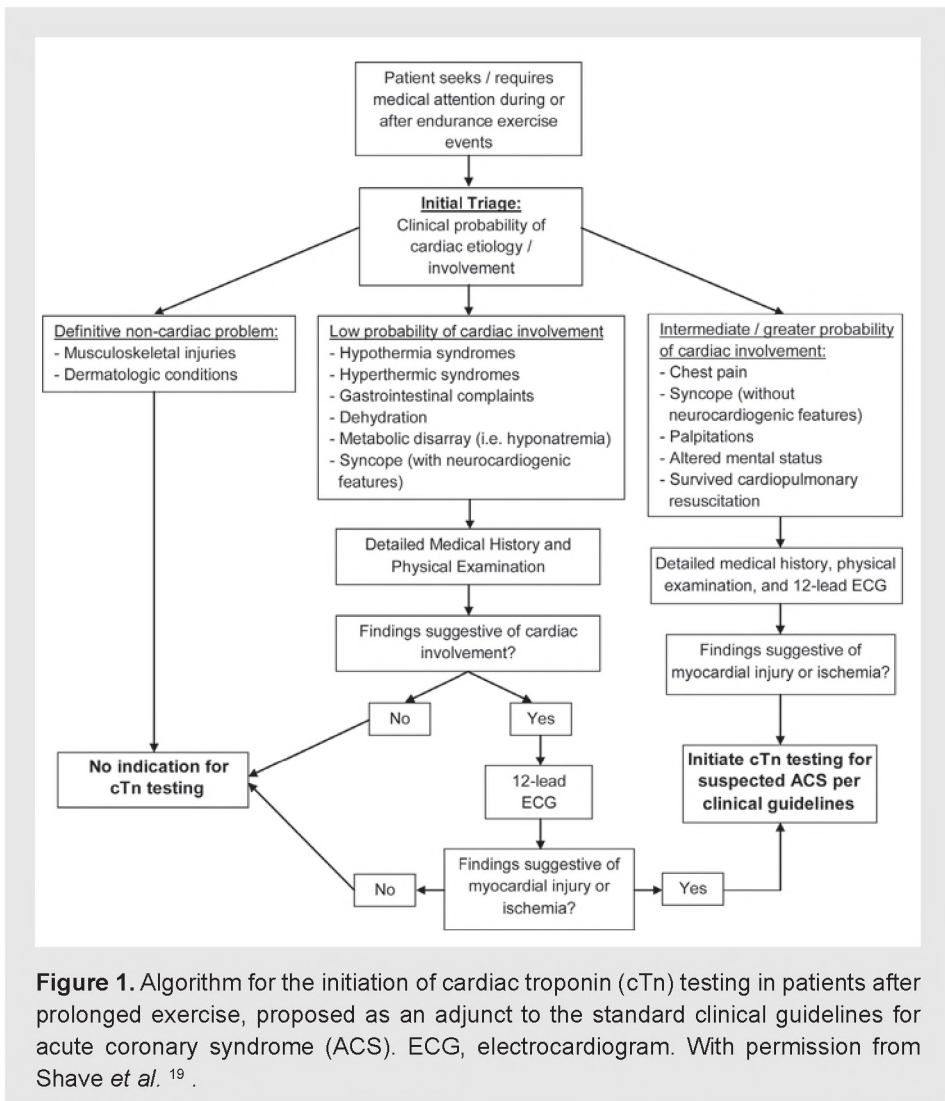
Parameter	Case 1	Case 2	Case 3
Exercise			
Type	walking	running	walking
Intensity	moderate - high	high	moderate
Duration	~7.5 h	1 h 12 min	~10.5 h
Cardiac troponins			
Assay	cTnI	cTnI	cTnI
cTn level (highest value)	0.448 µg/L	17.40 µg/L	4.26 µg/L
cTn > clinical cut-off	+	+	+
Physical complaints			
Chest pain	-	-	+
Sweating	-	-	-
Breathlessness	-	-	-
Nausea	-	+	-
Vomiting	-	-	-
Restlessness	-	-	-
Signs of ischemia			
ECG abnormalities	-	+	-
Cardiac imaging abnormalities	-	+	-
PCA	-	+	-

PCA: percutaneous coronary angiography, '-': not present, '+': present

symptoms as well as a rise/fall of cTn must be present for the diagnosis of AMI.^{5,}

¹⁸ Since no accompanying symptoms were present in Case 1, the increase in cTn is likely related to a physiological response during the exercise stimulus. Although walking is not regarded as either strenuous or exhaustive exercise, this subject exercised for 7.5 hours with an average heart rate of 160 bpm. Two recent studies have shown that a significant proportion of participants in long-distance walking events demonstrate an increase in cTnI, with 6-11% exceeding the AMI clinical cut-off.^{10, 15} These findings support a recent review that concluded that the exercise-induced increase in cTn in healthy individuals is a common phenomenon.¹⁹ Although the exact mechanism behind the physiological release of cTn during exercise is unknown, recent studies have hypothesized an increased membrane permeability of the cardiomyocytes following strenuous exercise.²⁰⁻²³ When the individual described in Case 1 was taken to hospital, assessment of blood cTn-levels could have caused confusion if clinicians had not appreciated

the importance of the recent 'exercise history', such as has been described in previous case reports.^{14, 24} It is worth noticing that only a mild increase in cTnI is observed in this subject after exercise. This "relatively" low cTnI, combined with a lack of clinical signs or symptoms suggestive for AMI, suggest a physiologic as opposed to a pathologic troponin release and as such does not warrant further medical attention.



On the other end of the spectrum, exercise can also act as a trigger for AMI. A crucial difference between Case 1 and 2 is the presentation of clinical symptoms that are strongly suggestive of AMI. According to a recently published algorithm for the differential diagnosis in participants who have recently completed unaccustomed exercise who then seek medical attention (Figure 1), cTn should be tested in this patient.¹⁹ While cTn levels were normal directly after admission to the hospital, cTnI was significantly elevated above the 99th percentile of the upper reference limit after two hours.⁵ In contrast to healthy subjects who usually demonstrate a direct increase in cTn post-exercise, it takes ~2 hours to detect elevated cTn levels in patients with ischemic cardiac injury. This characteristic delayed increase of cTnI highlights the importance of assessing clinical symptomatology alongside humoral markers.²⁵ Given the magnitude and kinetics of the subsequent cTnI release in Case 2, it is clear that this cannot be explained by the prior exercise alone, but is likely the direct result of cardiac damage. Indeed, we found classical clinical signs for AMI and characteristic changes in the 12-lead ECG, whilst percutaneous coronary angiography was necessary to open the right coronary artery. This case is, therefore, representative of an AMI, which in occurred after a bout of exercise.

Case 3 raises a number of questions, and exemplifies the difficulty of interpreting elevated cTn levels after prolonged exercise. Immediately after hospital admission, and also on subsequent days, cTn levels were above the clinical cut-off value. Furthermore, a “tight band feeling” was reported by the patient at admission. According to current guidelines,^{5, 8} the elevated cTn levels in combination with the clinical symptoms suggest the presence of an AMI. However, no signs of cardiac ischaemia or damage were found with additional testing. First, the clinical symptoms typical for a myocardial infarction (e.g. the “tight band feeling”) were temporary and disappeared within 1 hour 30 minutes. Second, the clinical investigations that were performed after admission (ECG recordings and echocardiography), and play a central role in the final diagnosis of AMI, were all negative and did not reveal evidence of ischaemic cardiac injury. Third, additional analysis performed 9 months after admission (resting ECG and cardiac MRI), showed no evidence for myocardial scarring or resting perfusion abnormalities. Taken together, the low cardiovascular risk profile and the absence of evidence of ischaemia, argue against the presence of a (meaningful) AMI.

Alternatively, the elevation in cTn levels may relate to the preceding exercise stimulus, such as observed in Case 1. However, the magnitude of cTn levels was substantially higher than typically reported in previous studies. Also, cTn levels remained elevated during the two days after exercise, whilst it can be expected that cTn will start to decline rapidly after exercise. Therefore, we believe it is unlikely that the elevated cTn levels were solely due to the walking exercise. Previous studies have reported that other (cardiovascular) pathologies are related to elevated cTn levels, such as pericarditis, myocarditis, pulmonary embolus, heart failure, renal

failure and sepsis.²⁶ These conditions should be taken into consideration when evaluating the high cTn levels in this patient. Given the temporal nature of physical complaints, normal ECG pattern after admission, absence of signs of dyspnea or hyperthermia, normal leukocyte concentration and mildly elevated CRP levels, it is unlikely that these (cardiovascular) pathologies can explain the elevation in cTnI in this subject. Finally, the elevated cTn levels in Case 3 might relate to the presence of a very small AMI. This limited ischemic region could explain the (modest) elevated troponin levels, whilst the size of this AMI would be too small to detect significant scarring or changes in cardiac function. Taken together, a final diagnosis is challenging for this subject. The elevation in cTnI in this subject unlikely relates to the preceding exercise bout, but also does not represent the typical AMI. Thus, this case highlights the clinical confusion that can occur with troponin testing in patients that have recently participated in exercise events.

Conclusion

Taken together, minor elevations in cTn in individuals admitted to hospital after strenuous exercise can sometimes be difficult to interpret as the minimal increase may relate to; 1. early release from necrotic tissue, and/or 2. a physiological exercise-induced stimulus. Before making a final diagnosis based on a positive cTn, physicians should take into account the magnitude and nature (transient/delayed) of cTn release, clinical symptoms as well as the (exercise) history of the individual.

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inlevere
rinet

Total body fluid (42 L)

Intracellular

Interstitium

Extracellular

Interstitium

Plasma

Cell body temperature

Distance
Grady
Interstitium

NaCl

Chapter 8

General discussion and future implications



The effects of endurance exercise on thermoregulation, fluid- and sodium balance and the cardiovascular system have been described frequently in participants of high intensity exercise events, such as a marathon or (Ironman) triathlon. In contrast, the physiological responses of prolonged, moderate intensity exercise, such as hiking or walking, are less well characterized. Given the large differences in subject characteristics of participants (e.g. age, physical fitness, health status) as well as differences in nature of exercise (e.g. duration, exercise intensity), extrapolation of current knowledge to walking marches is extremely difficult. Therefore, we explored the physiological demands of prolonged walking in this thesis in general, and focusing on the thermoregulation, fluid- and sodium balance and cardiac troponin release specifically. In this final chapter, our results are summarized and the consequences of our findings will be discussed and related to other studies.

Thermoregulation

Metabolic heat production increases immediately at the onset of exercise, as ~80% of the substrate turnover appears as heat.¹ During early stages of exercise, the rate of heat production exceeds the rate of heat dissipation, resulting in storage of undissipated heat, causing a modest and harmless rise in core body temperature. The increase in core body temperature importantly depends on exercise intensity, exercise duration, ambient conditions, fluid balance, and subject characteristics.²⁻⁴ Depending on these factors, core body temperature may reach a steady state (heat production and dissipation are balanced) or could result into uncompensable heat stress. In this latter circumstance, core body temperature becomes a limiting factor for optimal exercise performance,⁵ and may even result in development of heat illness.^{2, 6, 7}

Prolonged walking

To assess the thermoregulatory responses of participants of the Four Days Marches, we have pooled the data of the 2007 (n=63), 2008 (n=99), 2009 (n=100) and 2010 (n=99) editions, which was collected on all 4 days of the event. Our subjects demonstrated a baseline core body temperature of 37.3°C. After the onset of exercise, a relatively large rise in core body temperature was observed during the first 5 km, followed by a slow, but gradual increase thereafter (*Figure 2 at Chapter 4*). An average maximum core body temperature of $38.2 \pm 0.4^\circ\text{C}$ was reported during walking. This thermoregulatory response is comparable with participants of an Ironman triathlon (38.2°C),⁸ but significantly lower than subjects running a marathon under comparable conditions ($38.5 - 39.0^\circ\text{C}$).⁹⁻¹² Differences in exercise intensity across these different types of endurance exercise most likely explain this finding. Indeed, exercise intensity, which primarily determines the magnitude of metabolic heat production, is significantly related to the amount of heat accumulated in the body during exercise (Table 1).^{13, 14} Taken together,

prolonged walking is associated with a modest increase in core body temperature.

Subject and exercise characteristics

Although the mean core body temperature demonstrated a modest increase, at the individual level a large variation was observed (Figure 1). This large variation suggests that various parameters may contribute to the individual thermoregulatory response. In *Chapter 3* we demonstrated that pre- and postmenopausal women have slightly higher maximum core body temperatures than men. Nonetheless, the *increase* in core body temperature was comparable. In contrast, lean participants showed a lower rate of increase in core body temperature than overweight and obese participants (*Chapter 4*), despite a similar relative exercise intensity across these groups.

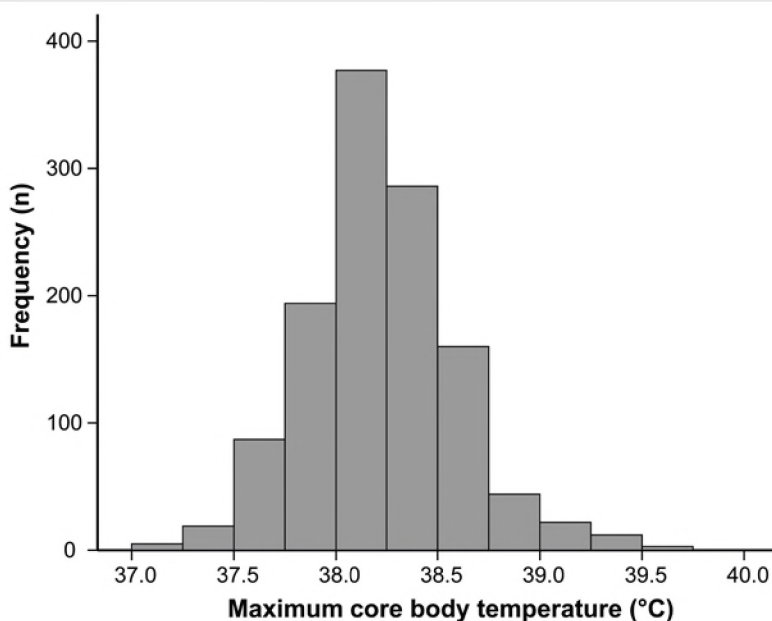


Figure 1. Frequency distribution of the maximum core body temperature in subjects that participated in the Four Days Marches studies between 2007 and 2010 (n=1209 observations).

Another interesting observation is that core body temperature did not reach a stable plateau in our subjects during prolonged moderate-intensity walking exercise. This suggests that the duration of the exercise stimulus contributes to the maximum core body temperature during exercise. Indeed, additional analysis confirmed that the maximum core body temperature depends on the distance

walked, with a higher core body temperature observed after walking a larger distance (Table 1). A potential confounder in this analysis is age, as the distance that subjects walk is largely based upon age. However, the maximum core body temperature did not differ across age groups ($p=0.18$, Table 1). Although it was suggested that ageing is related to a declined tolerance for heat stress,¹⁵ other factors than age itself (BMI, physical fitness, thirst and sweat response) more likely contribute to these observations in our subjects that performed walking exercise.¹⁶⁻¹⁹ Taken together, we found that sex, BMI, exercise intensity and distance category contribute to the maximum core body temperature during prolonged walking. However, we should emphasize that these differences are only subtle and that potentially other factors are involved in the development of thermoregulatory disorders.

Table 1. Maximum core body temperature for distance, age and exercise intensity groups. Values are presented as mean \pm standard deviation.

Parameter	Maximum T_c ($^{\circ}\text{C}$)	P-value
Exercise intensity		<0.001
< 60%	38.1 \pm 0.4	
60 – 70%	38.2 \pm 0.3	
70 – 80%	38.3 \pm 0.3	
> 80%	38.4 \pm 0.4	
Distance		<0.001
30 km	38.1 \pm 0.3	
40 km	38.2 \pm 0.4	
50 km	38.3 \pm 0.4	
Age*		0.18
<30 years	38.2 \pm 0.3	
30 – 39 years	38.4 \pm 0.4	
40 – 49 years	38.3 \pm 0.4	
50 – 59 years	38.2 \pm 0.4	
>60 years	38.3 \pm 0.4	

* To assess the effect of age over a large range, only the subjects that walk 50 km a day are selected for this analysis.

Ambient conditions

Air temperature, relative humidity, wind speed and solar radiation have a direct effect on the mechanisms of heat loss and heat gain. As ambient temperature rises, the effectiveness of heat loss by physical transfer (conduction and radiation) becomes compromised, leading to increased dependence on sweating and evaporation of water from the skin surface.¹ A

common method to determine the environmental heat stress is calculation of the Wet Bulb Globe Temperature (WBGT, see *Chapter 1*).²⁰ The risk to develop heat-related illnesses is suggested to have a linear relationship with the WBGT, whilst it is recommended to stop exercise with WBGT temperatures above 28.0°C.² To assess the influence of WBGT on thermoregulatory responses during prolonged walking, we followed a group of subjects (n=20) longitudinally during 4 subsequent Four Days Marches (2007, 2008, 2009 and 2010). Interestingly, maximum core body temperature did not differ across a WBGT between 17.6°C and 26.5°C (Figure 2). The observation that the human thermoregulation is able to maintain the core body temperature within a narrow range during exercise over a relatively large range of ambient temperatures has been described previously.²¹ Within this so-called 'prescriptive zone', the magnitude of the increase in core body temperature is proportional to the metabolic rate and independent of ambient temperatures.²¹ We found that exercise intensity in these 20 subject correlated significantly with the core body temperature ($R=0.26$, $P<0.001$). These data emphasize that core body temperature was well regulated in participants of the Four Days Marches, without any case of exertional hyperthermia or heat-related illnesses. Therefore, prolonged walking exercise in ambient conditions with a WBGT between 17.6°C and 26.5°C is performed with keeping core body temperature within normal and safe limits.

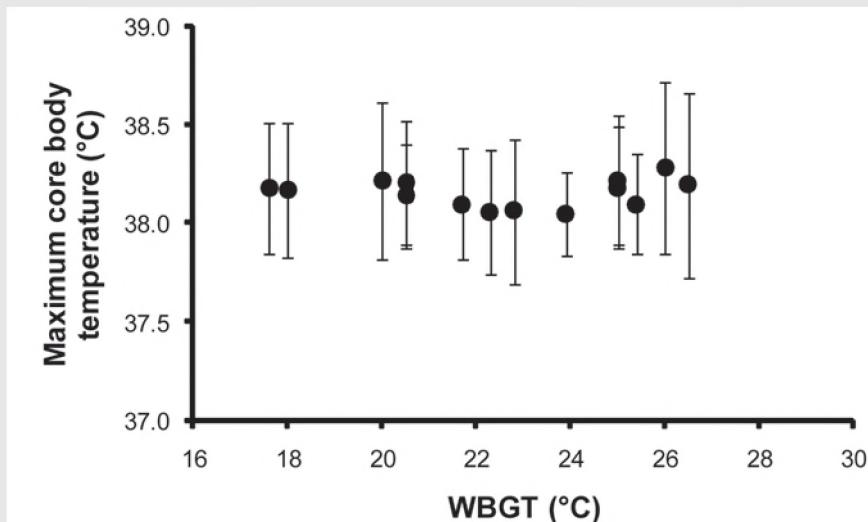


Figure 2. Maximum core body temperature with standard deviation under different ambient conditions (n=20). A variation in wet bulb globe temperature (WBGT) between 17.6°C and 26.5°C did not affect the thermoregulatory responses during prolonged walking.

Fluid and sodium balance

Prolonged exercise represents a strong stimulus for alterations in the balance between fluid intake and fluid output. Dehydration, hyponatremia or hyponatremia can develop, which may impact performance levels and general health.^{22, 23} We aimed to improve our insight into the effects of prolonged walking on fluid and sodium balance by the assessment of several parameters. Although fluid and sodium responses within the total group of subjects were within normal physiological ranges (body mass: -1.2%, plasma volume: -1.2%, sodium concentration: 141.9 mmol/L), a relatively large part of the participants demonstrated changes beyond normal limits. After a prolonged bout of walking exercise, 21% of the participants demonstrated dehydration, 13% reported hyponatremia and a small, but clinically relevant portion of the participants even developed hyponatremia (2%, *Chapter 2*). This indicates that, in contrast with the relatively modest changes in thermoregulation, prolonged moderate-intensity exercise importantly alters fluid and sodium balance.

Groups at risk

One of the aims of this thesis was to identify subjects who, based on individual or exercise characteristics, have an increased risk to develop fluid and sodium imbalance. In *Chapter 3* we showed that men demonstrated larger decreases in body mass and a 4 times higher risk to develop dehydration after prolonged exercise than women. We also demonstrated that obese subjects have higher sweat rates, lower urine outputs and a higher fluid intake compared to their lean peers (*Chapter 4*). In addition to men and obesity, previous studies reported that also sedentary elderly are prone to develop dehydration due to their diminished thirst response.^{16, 24, 25} Moreover, aging also causes a slower response to water or saline loads as the progressive fall of functioning nephrons leads to a lower glomerular filtration rate.^{26, 27} High rates of fluid ingestion may, therefore, increase the risk to develop hyponatremia in older participants. Indeed, subjects with post-exercise hyponatremia (n=13) were significantly older than subjects with normonatremia (66 and 56 year old respectively, $p < 0.05$). Alternatively, the use of medications like diuretics or nonsteroidal anti-inflammatory agents during exercise may impact the fluid and electrolyte balance.^{23, 28} Future experiments should further improve insight into the physiological responses during prolonged walking within these specific groups. This is of special importance since current fluid replacement guidelines only provide rather general recommendations,^{22, 29-31} and, therefore, are not suitable for a heterogeneous population like participants of the Four Days Marches. Better physiological insight into the fluid and electrolyte balance responses in these groups contribute to the optimization of fluid replacement advices before, during and after prolonged (walking) exercise.

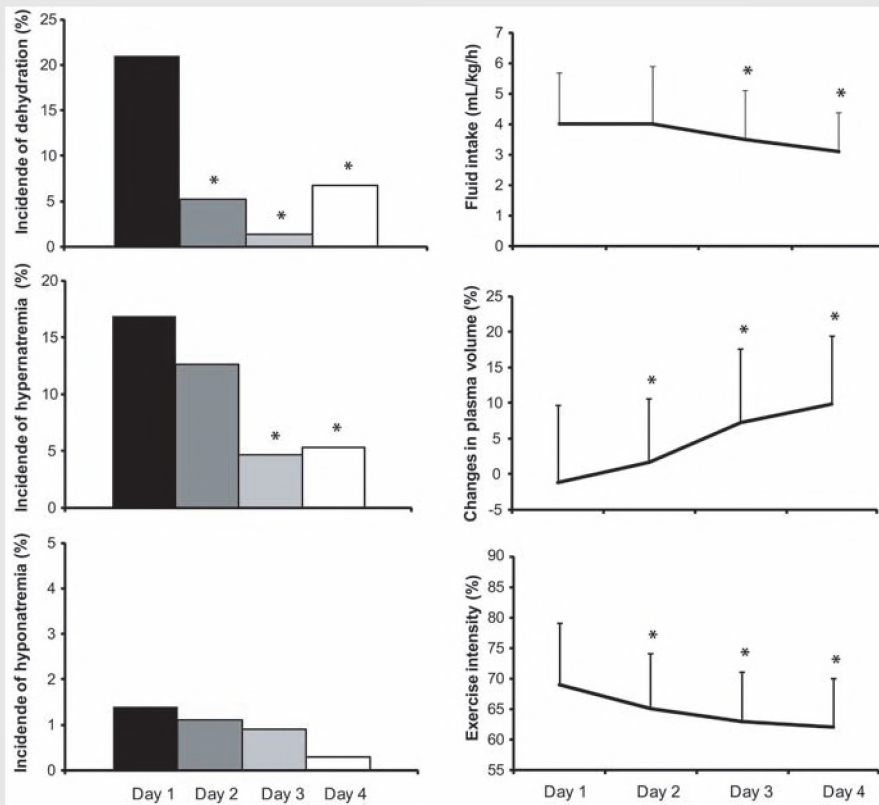


Figure 3. A significant decrease in the risk to develop fluid- and sodium disturbances is observed across days in a heterogeneous group of participants of the Four Days Marches. Whilst fluid intake and exercise intensity significantly decrease, plasma volume gradually increases over consecutive days. Data are means \pm standard deviation. *Significantly different from Day 1 ($n=353$).

Effects of exercise on consecutive days

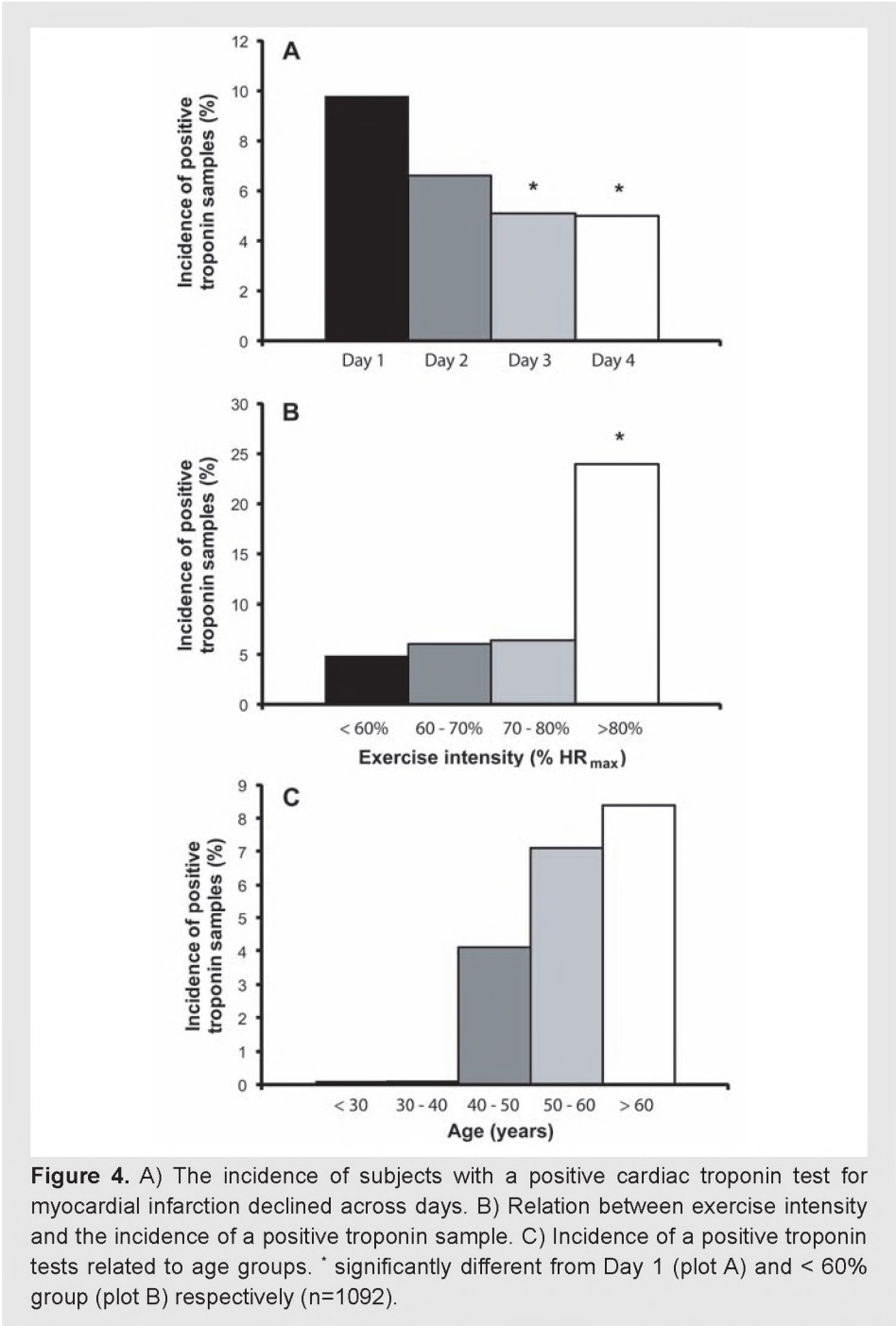
In contrast to most field studies that typically examined athletes before and after a single bout of exercise, we have examined subjects on 4 consecutive days. To exclude potential interference of exercise on subsequent days in our primary outcome parameters, *Chapter 2, 3 and 4* focused on the physiological responses of prolonged exercise on the first day of the Four Days Marches only. Here, we will examine whether our primary outcome parameters demonstrate changes across the 4 days of prolonged moderate-intensity exercise and, therefore, whether our conclusions can be extrapolated to the other walking days. Interestingly, and somewhat in contrast to our expectations,

physiological responses to prolonged exercise differ on consecutive days (Figure 3). The incidence of dehydration shows a significant drop after the first day ($p < 0.001$), and a comparable decrease is observed in the incidence of hyper- and hyponatremia. Although one may suggest that the drop in fluid and sodium disturbances is related to an increased fluid intake, a decline in fluid ingestion is observed across days (Figure 3). Moreover, ambient conditions and walking speed are comparable across days and, therefore, unlikely to explain these results. Alternatively, the attenuated risk may relate to changes in plasma volume. Previous studies showed that prolonged aerobic endurance exercise will cause an immediate decrease in plasma volume post-exercise, followed by an increase above baseline levels within 24 hours after exercise.³²⁻³⁴ Indeed, we found a decrease of -1.4% on the first day, whilst the plasma volume gradually increased to 9.8% on the fourth day. Although exercise is performed on subsequent days, which may interfere with the post-exercise change in plasma volume *per se*, this suggests that adaptations in plasma volume occur when performing prolonged walking exercise on multiple days. The increased plasma volume results in a larger buffer of the vascular compartment, which attenuates the risk for fluid and sodium disturbances.

We also observed a decrease in exercise intensity of 69% on day 1 to 62% of the maximal predicted heart rate on day 4, despite the fact that walking speed was maintained at the same level. The increased plasma volume may increase stroke volume and consequently (assuming a constant cardiac output) decrease heart rate. Whilst previous studies predominantly focused on single day athletic events, our findings provide novel insight into the physiological adaptations and risk assessment during prolonged exercise on consecutive days. The conclusions of *Chapters 2, 3 and 4*, which are based on the physiological responses on the first day of the Four Days Marches, cannot be extrapolated to the second, third and fourth day of this event. Hence, our data suggests that performing endurance exercise on subsequent days results in a lower risk for fluid and sodium disturbances, most likely attributable to changes in plasma volume.

Cardiac troponins

The release of troponins from the cardiac muscle cells is usually related to cardiac damage.³⁵ Detection of a rise and fall in cardiac troponins, therefore, plays a central role in the diagnosis of a myocardial infarction.^{36, 37} However, many studies in the past decade showed that high-intensity endurance exercise can elevate cardiac troponin levels above the clinical cut-off value in healthy athletes.³⁸⁻⁴⁴ In this thesis we showed that the exercise induced elevations in troponin levels is not restricted to high-intensity exercise only, but is also present in subjects that perform prolonged moderate-intensity exercise (*Chapter 5 and 6*). Participants of the Four Day Marches demonstrate elevated troponin levels



on all days, whilst 6-11% exceeds the clinical cut-off value for the diagnosis of a myocardial infarction. Our findings support a recent review that concluded that the post-exercise increase in cardiac troponin levels is a common phenomenon in healthy individuals.⁴⁵

Factors that influence troponin response

As elevated troponin levels in athletes can easily lead to a false-positive diagnosis and subsequent unnecessary medical treatment, we discussed the clinical management of exercise induced troponin elevations extensively in *Chapter 7*. For optimal differential diagnosis it is important to gain more insight in factors that contribute to the magnitude of the troponin response. We demonstrated in *Chapter 6* that exercise intensity is related to the increase of troponin levels. These findings are in agreement with a recent paper that found that exercise intensity influenced the magnitude of the troponin response.⁴⁶ Since our subjects demonstrated a decrease in exercise intensity across days (Figure 3), this might influence the incidence of subjects with a positive troponin test. Indeed, the incidence of positive troponin tests declined over consecutive days and depends strongly on the exercise intensity (Figure 4). These findings reinforce the observation that the magnitude of the troponin response is higher in marathon races (i.e. prolonged high-intensity) compared to ultra endurance races (i.e. moderate to high-intensity) and prolonged walking (i.e. moderate-intensity).^{45, 47}

We found that age, walking speed and cardiovascular pathology contribute to the exercise induced troponin response (*Chapter 5*). However, exercise intensity did not correlate with the age of our participants ($r=0.05$, $p=0.08$). The increased incidence in older subjects is therefore more likely to be attributed to other factors. A potential explanation may be due to a decreased kidney function in elderly,⁴⁸ and their subsequent lower clearance of cardiac troponins from the circulation. Alternatively, the age-dependent increase in cardiovascular pathology might also contribute to the higher incidence in older subjects. Future studies should further elucidate the difference in the magnitude of troponin responses between young, middle-aged and old subjects. These findings could help physicians and researchers to better understand the role of exercise induced elevations in troponin levels. Currently it is known that exercise intensity and age, but also walking speed and cardiovascular pathology, are related to a larger increase in troponin levels.

Mechanisms

Based on our interesting and novel findings, one may question the underlying (patho)physiological mechanisms for this increase in cardiac troponin during exercise. Potentially, elevated troponin levels are related to an increased membrane permeability, which can be due to increased mechanical stress, oxidative radicals, altered acid base balance or stimulation of integrins.⁴⁹⁻⁵² The leaky cell membrane allows unbound cardiac troponins in the cytosolic pool (<10%) to

enter the circulation.⁵³ The post-exercise elevated troponin levels could therefore be due to passive diffusion from the intra- to the extra-cellular compartment. A different hypothesis for the elevated cardiac troponin levels relates to an impaired clearance of troponin from the circulation. It is well known that blood flow in the kidneys and abdominal area reduces markedly during exercise.^{54, 55} Consequently, the kidneys have an attenuated capacity to secrete substances (e.g. the troponin complex), which may contribute to the elevated troponin levels in the circulating blood during prolonged exercise. Another mechanism to explain the increased troponin levels is associated with the development of “bubbles” (blebs) on the surface of the cardiac cell.⁵⁶ These blebs develop from the plasma membrane in response to temporary ischemia. When re-oxygenation occurs timely, the blebs are reabsorbed or shed into the circulation. During severe or prolonged ischemic conditions, the blebs grow and then collapse. This model suggests that exercise-related cardiac ischemia does not induce irreversible myocardial injury, but triggers the shedding of cardiac blebs which leads to increased levels of cardiac troponins.⁵⁶ A last (pathological) mechanism that could explain elevated troponin levels after exercise is myocardial cell necrosis. It is possible that post-exercise increased troponin levels relate to direct myocardial damage with cell death during exercise. Irrespective of the cause of troponin-release, the magnitude and the kinetics of troponin levels are importantly different between athletes after a strenuous exercise bout and patients suffering from a myocardial infarction. Whilst troponin release in athletes shows a biphasic response and only a moderate elevation,⁵⁷ patients with ischemic cardiac injury usually demonstrate troponin levels which are multiple times above the clinical cut-off value and remain above this value for 4 to 10 days.⁵⁸ Future studies should further examine whether post-exercise cardiac troponin release relates to a physiological or a pathophysiological response, thereby helping clinicians to improve their decision making around these positive troponin tests.

Future directions

Participants of the Four Days Marches were able to regulate their core body temperature properly, however, a relatively large part of our subjects demonstrated fluid (21%) or sodium disturbances (15%). Extrapolating these results to the entire field of participants of the event (n=40,000) suggests that dehydration (n=8,400), hypernatremia (n=5,200) and hyponatremia (n=600) may be a greater problem than previously recognized. Nonetheless, only ~500 participants usually drop out during the first day of this walking event, often because of problems related to muscles and joints. Whilst this questions the clinical impact of fluid and sodium disturbances, previous case studies have demonstrated the detrimental health effects of large changes in fluid and sodium disturbances.

Due to the lack of a gold standard to measure hydration status, the definition of

dehydration is currently subject of ongoing debate.⁵⁹⁻⁶² Although a loss of more than 2% of the body mass is frequently used for dehydration in humans, this threshold is arbitrary and might not be applicable to the heterogeneous population that participates in exercise events nowadays. Therefore, more insight is needed into the individual responses to different levels of dehydration in field conditions. These findings can help to develop personalized fluid replacement guidelines during exercise. In addition, this may reduce the number of disturbances and prevent health problems and/or performance loss.

Another important finding in this thesis is that elevated troponin levels after prolonged walking is a general finding in our subjects. Although the magnitude of this response is small, 6 – 11% of the subjects showed values above the clinical cut-off value for diagnosis of a myocardial infarction. Several studies investigated the myocardial function in athletes with elevated troponin levels. Whilst most studies did not find a relation between post-exercise troponin levels and permanent myocardial injury using noninvasive imaging techniques (MRI or echocardiography),⁶³⁻⁶⁵ myocardial fibrosis and late gadolinium enhancement were reported in older endurance athletes.^{66, 67} These inconsistent findings emphasize the need to further unravel the mechanisms of exercise-induced troponin release. Furthermore, the long term effects of post-exercise troponin elevations on general health, and cardiovascular pathology in specific, are currently unknown. Potentially, a large release of cardiac troponins after exercise has a prognostic value for the future development of cardiac problems, as this could reveal increased sensitivity for the cardiac muscle damage. A long-term cohort study could address this topic.

A last perspective is the development of technical devices that facilitate scientists, physicians and organizations to real-time monitor physiological parameters like core body temperature, sodium balance and the level of dehydration in all subjects of large exercise events. Whilst the basic techniques for these future products are already available (e.g. GPS, GSM, RFID chips and internet databases), this has not been applied to the field of exercise (science). These tools can help physicians and organizations to monitor the safety of participants and to respond adequately if necessary. Furthermore, these novel applications enable the online monitoring of physiological responses during prolonged exercise and may bring the field of exercise science to the next level.

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Click to - and produce > 100 Pa

Total body fluid (42 L)

Intracellular

Interstitium
10.0 L

Extracellular

Interstitium
10.0 L

Plasma
3.0 L

Cell body temperature

Distance
Grain
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Summary

Nederlandse samenvatting

Summary

Walking is one of the most popular types of leisure time physical activities, and is often prescribed to patients to improve their health and to prevent (co) morbidities. Although walking is commonly regarded as a safe type of exercise, recent case studies have reported thermoregulatory disorders and sodium disturbances in healthy individuals after completing prolonged walking exercise. Since current knowledge about physiological responses of prolonged exercise is limited to young, healthy and well-trained subjects, extrapolation of these findings to the heterogeneous population that participates in organized walking marches is extremely difficult. Therefore, the general aim of this thesis was to assess the responses of core body temperature, fluid- and sodium balance and cardiac troponins in a large heterogeneous group of subjects participating in the International Nijmegen Four Days Marches.

Chapter 1 deals with the physiological principles of 1) human thermoregulation, 2) fluid and sodium balance, and 3) cardiac troponin complex. The effect of physical exercise on these 3 systems is discussed extensively. Furthermore, thermoregulatory disorders and fluid and sodium disturbances are presented, with special attention to the effects on the athlete's health status. At the end of this first chapter, the purposes and hypothesis of the individual chapters are presented, followed by the experimental model and measurement techniques that have been used in this thesis.

In **Chapter 2** we explored the physiological responses of prolonged walking exercise in a large heterogeneous group of subjects. During 8.5 hours of prolonged moderate-intensity walking exercise in temperate ambient conditions, core body temperature increased modestly, while the magnitude of this increase was related to exercise intensity. More importantly, a large group of subjects (15%) demonstrated a post-exercise sodium disturbance. Given the strong interaction between the fluid and sodium balance, it was suggested that participants of walking events may also be prone to dehydration. Although prolonged walking exercise is well tolerated by subjects of various ages and health conditions, it represents a considerable physiological strain on the human body.

The role of sex in the regulation of fluid balance responses during exercise is examined in **Chapter 3**. A clear sex difference was observed, with men demonstrating a significantly lower fluid intake, higher body mass loss, higher incidence of dehydration, larger increase in plasma sodium levels and a higher incidence of hyponatremia. This sex difference was reinforced by statistical analysis, which revealed that sex and fluid intake were the only parameters that correlate with the presence of dehydration. These findings suggest that the control of fluid balance during prolonged walking exercise is regulated differently in men and women.

As prolonged, moderate-intensity exercise training is routinely prescribed to subjects with obesity, and obesity may alter thermoregulation and fluid balance, we compared the physiological responses of prolonged walking among lean, overweight and obese subjects in **Chapter 4**. Although exercise intensity and duration was comparable across groups, obese subjects demonstrate a larger change in markers of fluid and sodium balance than their lean counterparts during prolonged moderate-intensity exercise. These findings suggest that overweight and obese subjects, especially under strenuous environmental conditions, have an increased risk to develop fluid and sodium disturbances.

In **Chapter 5** we examined cardiac troponin I levels at baseline and immediately post-exercise after each day of the Nijmegen Four Days Marches. Troponin levels significantly increased after the first day, but increased also on consecutive days after a comparable bout of walking exercise. Furthermore, we showed that age, speed and the presence of cardiovascular pathology were significantly related to post-exercise troponin levels. In total, 6% of our subjects demonstrated a troponin level above the clinical cut-off value for myocardial infarction on 1 or more days. As these participants were older, walked at a higher relative exercise intensity, and reported a higher prevalence of cardiovascular pathology than subjects with a negative troponin test, these findings reinforced our previous analysis. In conclusion, prolonged moderate-intensity walking exercise causes an increase in cardiac troponin levels in a non-athletic population.

The effect of obesity on cardiac troponin release after prolonged moderate-intensity walking exercise is discussed in **Chapter 6**. Cardiac troponin levels significantly increased after exercise, while this response was not different between lean, overweight and obese subjects. In 11 participants, troponin levels were elevated above the clinical cut-off value for myocardial infarction. Logistic regression analysis identified exercise intensity ($P < 0.001$), but not BMI, body fat percentage or waist circumference to significantly relate to positive troponin tests. Therefore, measures of obesity unlikely relate to the magnitude of the post-exercise elevation in cardiac troponin I.

Chapter 7 deals with the difficult clinical interpretation of positive troponin tests in people who have recently undertaken exercise. The presentation and discussion of three distinct case studies, each with different symptoms and a different final diagnosis, emphasizes that a detailed assessment of all symptoms and a thorough patient-history are prerequisite for accurate interpretation of a positive cardiac troponin test. This information can help clinicians and laboratories to improve the clinical management of a positive troponin test.

In **Chapter 8** the current knowledge on the physiological demands of prolonged exercise was reviewed and discussed from previous studies and available data from this thesis. Combining our data collected during the 2007, 2008, 2009 and

2010 FourDays Marches allowed us to examine the influence of ambient conditions on physiological responses. This unique analysis allowed us to take our data to the next level, which was not possible when analyzing the data from the individual years. For example, we found that maximum core body temperature did not differ across a wet bulb globe temperature between 17.6°C and 26.5°C. Furthermore, we performed the novel and clinically relevant comparison regarding the impact of prolonged walking on a single day *versus* multiple days. Surprisingly, the incidence of subjects with a fluid or sodium disturbance declined markedly across days, possibly due to an immediate increase in plasma volume during the event. This suggests that the risk for fluid and sodium disturbances, but also elevations in cardiac troponin, decline when performing walking exercise on multiple days. Finally, we speculated in this last chapter about potential mechanisms that can explain the exercise induced elevations in troponin levels.

Nederlandse samenvatting

Wandelen is in veel landen één van de meest populaire vrijetijdsbesteding. De belangrijkste redenen hiervoor zijn dat het toegankelijk is voor jong en oud, en dat men eenvoudig de intensiteit van deze inspanning kan reguleren. Daarnaast wordt wandelen vaak door artsen voorgeschreven als een effectieve manier om de gezondheid van patiënten te verbeteren. Ondanks dat wandelen over het algemeen wordt beschouwd als een veilige manier van inspanning, laten recente studies in de medische literatuur zien dat er ook gezondheidsrisico's kunnen optreden. Zo zijn verstoringen van de temperatuurregulatie (oververhitting), water- en zoutbalans (hyponatriëmie; een te lage zoutconcentratie) tijdens extreme omstandigheden gerapporteerd. Een voorbeeld hiervan is de Nijmeegse Vierdaagse van 2006. Tijdens deze wandeltocht zijn diverse deelnemers in het ziekenhuis beland met onder andere hittegerelateerde klachten. Aangezien de huidige beschikbare kennis over de fysiologische reacties tijdens duurinspanning zich voornamelijk beperkt tot jonge, gezonde en zeer getrainde personen, is vertaling van deze resultaten naar de gemiddelde, normale wandelaar onmogelijk. De populatie van wandelevenementen betreft namelijk een bijzondere samenstelling. Deelnemers variëren vaak van van jong tot oud, getraind of ongetraind, tot mensen die al zelfs een ziekte onder de leden hebben. Om deze reden richt dit proefschrift zich op het bepalen van de lichaamsreacties op de kerntemperatuur, water- en zoutbalans en cardiaal troponine in een grote heterogene groep wandelaars die deelneemt aan de Nijmeegse Vierdaagse.

In **Hoofdstuk 1** worden de diverse fysiologische principes van 1) de thermoregulatie, 2) de water- en zoutbalans, en 3) het cardiale troponine complex uiteen gezet. Tevens wordt het effect van lichamelijke inspanning op deze drie systemen uitgebreid besproken. Speciale aandacht gaat hierbij uit naar de verstoringen van de thermoregulatie, water- en zoutbalans, en het effect daarvan op de gezondheid van een individu. Aan het einde van dit hoofdstuk worden de doelen en hypothesen van alle afzonderlijke hoofdstukken besproken, gevolgd door de onderzoeksopzet en meetmethoden die zijn gebruik in dit proefschrift.

Om de fysiologische reacties tijdens langdurig wandelen nader te onderzoeken hebben we in **Hoofdstuk 2** een groep van 63 deelnemers tijdens de Nijmeegse Vierdaagse van 2007 gevolgd. Door middel van het uitlezen van de temperatuurpil die door deze groep wandelaars werd ingeslikt, en het prikken van bloed was het mogelijk om de thermoregulatie en de zoutbalans in kaart te brengen. Tijdens de wandeling met een matige intensiteit, die gemiddeld 8,5 uur duurde, en een omgevingstemperatuur van 22,3°C, nam de kerntemperatuur toe tot een maximum van 38,0°C. De mate van temperatuurstijging bleek samen te hangen met de inspanningsintensiteit. Een belangrijkere bevinding was echter dat een relatief grote groep deelnemers een verstoorde zoutbalans liet zien na de eerste wandeldag: in 13% van de wandelaars was de zoutconcentratie

in het bloed te hoog, terwijl 2% juist een te lage zoutconcentratie had. Gezien de sterke fysiologische interactie tussen de water- en zoutbalans, is het zeer waarschijnlijk dat deelnemers aan wandelevenementen te kampen hebben met uitdroging. Deze bevindingen suggereren dat langdurig wandelen zorgt voor een substantiële fysiologische belasting op het menselijk lichaam, ondanks dat het merendeel van de deelnemers deze inspanning prima kan verdragen.

De invloed van het geslacht op de reacties van de waterbalans tijdens duurinspanning wordt besproken in **Hoofdstuk 3**. Hoewel voorgaande onderzoeken hebben aangetoond dat de zweetproductie verschilt tussen mannen en vrouwen, is er nog nooit onderzoek uitgevoerd waarin de vochtbalans van beide geslachten met elkaar werd vergeleken tijdens inspanning. In dit hoofdstuk tonen we aan dat mannen een significant lagere vochtinname rapporteerden, meer lichaamsgewicht verliezen tijdens inspanning, vaker uitgedroogd zijn (34% tegenover 12%), een grotere toename van de natriumconcentratie hebben en daardoor vaker hypernatriëmie vertonen (27% tegenover 0%). Dit geslachtsverschil wordt bovendien versterkt door een aanvullende statistische analyse waarin geslacht en vochtinname als enige twee parameters een relatie tonen met het optreden van uitdroging. Deze gegevens wijzen erop dat de waterbalans op een verschillende manier wordt gereguleerd tussen mannen en vrouwen tijdens langdurig wandelen.

Een andere parameter die mogelijk een groot effect heeft op de thermoregulatie, water- en zoutbalans tijdens wandelen, is de lichaamssamenstelling van de wandelaar. Wandelaars met overgewicht en obesitas hebben in vergelijking met slanke wandelaars een groter lichaamsoppervlak, meer zweetklieren en een grotere thermische isolatie door de aanwezige vetmassa. In dat kader hebben we in **Hoofdstuk 4** de fysiologische reacties vergeleken tussen slanke wandelaars, en deelnemers met overgewicht en obesitas. Hoewel de inspanningsintensiteit (~72% van de maximaal voorspelde hartfrequentie) en wandelduur (~8 uur en 41 minuten) vergelijkbaar waren tussen de 3 groepen, lieten deelnemers met obesitas een grotere variatie in markers van de water- en zoutbalans zien in vergelijking met slanke wandelaars. Naast een grotere vochtinname en zweetproductie, was de urine productie lager en het soortelijk gewicht van de urine hoger. Bovendien lieten wandelaars met overgewicht en obesitas een toename van de natriumconcentratie zien, waar deze bij slanke wandelaars niet veranderde. Tot slot werd er een sterkere toename van de kerntemperatuur in wandelaars met obesitas gevonden. Deze resultaten wijzen erop dat wandelaars met overgewicht en obesitas, voornamelijk tijdens extreme weersomstandigheden, een verhoogd risico hebben op het ontwikkelen van verstoringen van de thermoregulatie, water- en/of zoutbalans.

In **Hoofdstuk 5** onderzoeken we de concentratie troponine I voorafgaand aan, en na afloop van iedere wandeldag tijdens de Nijmeegse Vierdaagse. Normaal

gesproken wordt een troponine test uitgevoerd in het ziekenhuis om vast te stellen of er bij een patiënt hartspierschade is opgetreden. Echter eerdere studies hebben laten zien dat extreme duurinspanning, zoals een marathon of triatlon, ook kan leiden tot een toename van de troponine concentratie in het bloed. De studie die beschreven staat in dit hoofdstuk laat voor het eerst zien dat matig-intensieve duurinspanning voor een significante toename van troponine I zorgt. Deze verhoogde troponine waarden werden na afloop van alle 4 de wandeldagen gevonden. Vervolgens is bekeken welke variabelen gerelateerd kunnen worden aan de troponine I concentratie na het wandelen. Zowel leeftijd, wandelsnelheid en het hebben van een cardiovasculaire voorgeschiedenis hingen samen met de troponine concentratie. Een andere interessante bevinding uit deze studie was dat 6% van de deelnemers een troponine concentratie boven het klinisch afkappunt rapporteerde op 1 of meerdere dagen. Met andere woorden, op basis van de troponine concentratie zou een arts normaal gesproken bij deze personen de diagnose hartinfarct stellen. Aangezien deze groep deelnemers ouder was, op een hogere inspanningsintensiteit wandelde en vaker een cardiovasculaire voorgeschiedenis hadden dan de deelnemers met een concentratie beneden het klinisch afkappunt, bevestigen deze bevindingen onze eerder uitgevoerde analyse. Kort samengevat kan worden vastgesteld dat langdurig wandelen op een matige intensiteit een substantiële toename van de troponine I concentratie tot gevolg heeft.

Hoofdstuk 6 beschrijft vervolgens het effect van obesitas op de troponine I concentratie. Obesewandelaars hebben door hun lichaamssamenstelling namelijk een verhoogd risico op het ontwikkelen van cardiovasculaire aandoeningen. In dat kader zou de troponine respons in deze groep mogelijk anders kunnen zijn dan bij slanke wandelaars. Het bleek echter dat de significante toename van troponine I na langdurig wandelen niet verschillend was tussen slanke controle personen en wandelaars met overgewicht of obesitas. Dit werd bevestigd door de 11 wandelaars die een troponine I concentratie boven het klinisch afkappunt hadden. De body mass index (BMI), het vetpercentage en de buikomvang was vergelijkbaar met de groep wandelaars met een lage concentratie troponine. Uit deze gegevens kan worden geconcludeerd dat obesitas waarschijnlijk niet bijdraagt aan de stijging van de troponine I concentraties tijdens wandelen.

In *Hoofdstuk 5* en *6* is beschreven dat duurinspanning kan leiden tot een troponine concentratie die stijgt tot boven het klinisch afkappunt voor het aantonen van een hartinfarct. Zodoende is het voor klinici erg lastig om troponine testen te beoordelen van patiënten die zich voor opname in het ziekenhuis (langdurig) ingespannen hebben. In dit kader beschrijven we in **Hoofdstuk 7** drie casussen, waarbij diverse symptomen worden gerapporteerd en uiteindelijk drie verschillende diagnoses worden gesteld. Hierbij wordt benadrukt dat de beoordeling van de lichamelijke klachten, en een uitgebreide anamnese van essentieel belang zijn bij de interpretatie van een verhoogde troponine concentratie. De uitgebreide

discussie rondom deze drie casussen kan daarom bijdragen aan het verbeteren van klinisch management door artsen en laboratoria.

Het sluitstuk van dit proefschrift is **Hoofdstuk 8**. Hierin worden alle resultaten bediscussieerd in relatie tot de huidige kennis op het gebied van de inspanningsfysiologie. Daarnaast worden in dit hoofdstuk de data van de Vierdaagse onderzoeken in 2007, 2008, 2009 en 2010 gecombineerd om de invloed van omgevingstemperaturen op de fysiologische reacties van wandelaars in kaart te brengen. Hieruit blijkt dat de maximale kerntemperatuur niet verschillend is bij een 'wet bulb globe temperature' (een maat voor omgevingstemperatuur) tussen de 17.6°C en 26.5°C. Tevens is in dit hoofdstuk het verschil in fysiologische reacties tussen langdurig wandelen op 1 dag of op meerdere dagen bekeken. Het was opvallend dat het aantal wandelaars tijdens de Vierdaagse met een verstoorde water- of zoutbalans per dag afnam. Waarschijnlijk ligt een toename van het plasmavolume hieraan ten grondslag. Deze bevindingen duiden erop dat de risico's op fysiologische verstoringen afnemen tijdens een inspanning over meerdere dagen. Tevens wordt er in dit hoofdstuk dieper in gegaan op de inspanningsgebonden toename van de troponine concentratie. Hierbij zijn diverse mechanismen bediscussieerd die dit fenomeen mogelijk kunnen verklaren. Tot slot worden diverse suggesties voor mogelijk vervolgonderzoek besproken naar aanleiding van de bevindingen in dit proefschrift.

Click to add picture (max 9x)

Total body fluid (42 L)

Intracellular

Interstitium
10.0 L

Extracellular

Interstitium
10.0 L

Plasma
3.0 L

Core body temperature



[illegible]

36 posten langs de route, 1096 temperatuurpillen, 3241 plaszakken, 5280 hartfrequentieregistraties, 5710 epjes en 17770 milliliter bloed. Dergelijke statistieken horen bij een evenement als de Vierdaagse, en zijn in mijn geval het resultaat van 4 mooie jaren onderzoek. Voor deze prestatie ben ik veel mensen dankbaar die ik in deze epiloog graag zou willen bedanken.

Allereerst spreek ik mijn bewondering uit voor de 361 deelnemers die naast het volbrengen van de Vierdaagse ook nog de tijd, moeite en energie hadden om mee te werken aan alle metingen die wij ieder jaar weer bedachten. Specifiek wil ik daarbij de wandelaars bedanken die tijdens alle 4 de Vierdaagse onderzoeken hebben meegewerkt, en dit jaar de kans hebben om een lustrum te vieren. Het is prachtig om te zien dat velen van jullie 's ochtends ruim voor de start al naar het onderzoekscentrum komen om daar gezellig een kop koffie te drinken en bij te kletsen voordat er weer gepresteerd moet worden.

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inlevere
rinet

Total body fluid (42 L)

Intracellular

Interstitium

Extracellular

Interstitium

Plasma

Cell body temperature

Distance
Grady
Interstitium

NaCl

Curriculum vitae

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7. **T.M.H. Eijsvogels**, M.T.W. Veltmeijer, K. George, M.T.E. Hopman, D.H.J. Thijssen (2010) The impact of obesity on cardiac troponin levels after prolonged exercise in humans. *In revision* (Chapter 6)

Curriculum vitae

Thijs Eijsvogels werd op 16 juni 1984 geboren te 's-Hertogenbosch en groeide op in het nabij gelegen Lithoijen. Na het behalen van het Atheneum diploma aan het Titus Brandsma Lyceum te Oss, studeerde hij van 2002 tot 2007 Biomedische Wetenschappen aan de Radboud Universiteit Nijmegen. Voor zijn afstudeerrichting Bewegingswetenschappen liep hij zijn eerste wetenschappelijke stage op de Afdeling Fysiologie van het UMC St Radboud. Tijdens het Belgisch - Nederlands studentencongres der bewegingswetenschappen won hij de eerste prijs met de presentatie van de bevindingen van deze stage. Gedurende de master fase van zijn opleiding liep hij achtereenvolgens stage bij het KCMC Hospital in Moshi (Tanzania), Pandemonia Science Theatre te Amsterdam en tot slot bij TNO Defensie en Veiligheid in Soesterberg. Na het behalen van zijn bul keerde hij in november 2007 als promovendus terug op de afdeling Fysiologie. Onder leiding van Prof. Dr. Maria Hopman en Dr. Dick Thijssen richtte hij zich op het in dit proefschrift beschreven onderzoeken. Zijn eerste publicatie (Hoofdstuk 2) leverde hem een eervolle vermelding op voor de Jonge Auteurs prijs in het Nederlands Tijdschrift voor Geneeskunde. Naast het Vierdaagse onderzoek, heeft hij tijdens zijn promotie ook bij andere evenementen onderzoek verricht. Zo onderzocht hij de thermoregulatie van recreanten en professionals tijdens de Zevenheuvelenloop, het effect van een individueel vochtadvies op de vochtbalans bij deelnemers van de Marathon Eindhoven, en de variatie in kerntemperatuur tijdens een expeditie naar de top van de Mount Everest. Daarnaast heeft hij als algemeen lid plaats genomen in de PhD Council van het Nijmegen Centre for Evidence Based Practice (NCEBP). Behalve onderzoek naar de effecten van langdurig wandelen heeft hij ook zelf 2 grote wandelevenementen volbracht: de Oxfam Novib Trailwalker (100 km) en de 80 van de Langstraat (80 km). Op dit moment is hij nog steeds als onderzoeker verbonden aan de afdeling Fysiologie, alwaar hij naast zijn wetenschappelijke en onderwijs taken tevens als studentencoördinator fungeert.